The origin of a name that reflects Europe’s cultural roots.

**Ancient Greek**

αἷμα [haima] = blood  
αἷματος [haimatos] = of blood  
λόγος [logos] = reasoning

**Scientific Latin**

haematologicus (adjective) = related to blood

**Scientific Latin**

haematologica (adjective, plural and neuter, used as a noun) = hematological subjects

**Modern English**

the hematology journal  
2004 JCR® Impact Factor = 4.192

Haematologica/The Hematology Journal, as the official organ of the European Hematology Association (EHA), aims not only to serve the scientific community, but also to promote European cultural identity.
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Words of welcome

Welcome to Amsterdam, to the 11th Congress of the European Hematology Association. The Scientific Program Committee and the Scientific and Education Committee of the 11th Congress have developed a most attractive program with expert hematologists presenting their timely views in sessions of a different scope covering both benign and malignant hematology. You are invited to participate in high quality Education, Meet-the-Expert, Hematology-in-Focus and Plenary sessions, as well as in challenging Lunch Debates, Clinical Trial Updates and Science-in-Progress sessions.

From the large number of abstracts submitted a challenging program of simultaneous oral sessions and poster sessions has been established. The 6 best abstracts will be presented during the Presidential Symposium. This year, for the second time, a joint EHA-ASH Symposium will take place on Saturday, June 17. A number of meetings of EHA Scientific Working Groups will be held, which will be of interest to many of you.

In addition, 25 Satellite Symposia will run on Super Thursday, covering the State-of-the-Art in experimental and clinical hematology. The Joint Symposium of the European School of Haematology (ESH) and EHA will take place on Friday.

In contrast to previous meetings, the Opening Ceremony will now take place on Friday, June 16, directly followed by the presentation of the José Carreras Lecture by Professor Eliane Gluckman. In the same session, the winners of the EHA-José Carreras Foundation Young Investigator Fellowship and the additional EHA Fellowships and Grants will be presented. For the first time a press conference will be held dealing with major topics selected from the presentations given during the Presidential Symposium.

The congress program is accredited for Continuing Medical Education (CME) by the EHA-CME System, which is the new name for the European Council for Accreditation in Hematology (ECAH). Since this European Commission funded project ended in November 2005 EHA assures the continuity in collaboration with many of the original ECAH partners including ESH. EHA and ESH are dedicated to establishing modern hematology in the European curriculum for medical postgraduate education. The scientific program of the 11th Congress of the EHA has also been reviewed and approved for accreditation by the American Medical Association (AMA).

From a social point of view, it goes without saying that Amsterdam is unbeatable in June! The RAI Congress Centre is close to the city center and you should at least expose yourself to one museum (2006 has been appointed as the Rembrandt year). An extensive program for accompanying persons has been developed, visiting places of interest throughout the Netherlands.

Hand in hand with our meeting progressing will be the Dutch National Soccer Team finding its way to the final game in the World Championship Tournament in Germany taking place at the same time. We will have television screens throughout the conference center to keep you posted (not only on the achievements of the Dutch team).

Do not miss our social event in the former Stock Exchange of Amsterdam (1903), the “Beurs van Berlage” (www.beursvanberlage.nl), which takes place on Saturday night, June 17 from 20.30 hours till midnight. Although this year no full buffet dinner will be served, there are plenty of bites and drinks to get you through. An attractive evening program will be presented in a fascinating environment comprising music entertainment, dancing, a big screen showing a live soccer game from the World Championship Tournament in Germany (Italy vs. USA) and additional surprises.

On behalf of the EHA Board, the Scientific Program Committee and the Scientific and Education Committee of the 11th Congress: again welcome to Amsterdam. We trust that this number one hematology congress in Europe will provide you with intense interaction with your peers and induce new creative ideas for your work!

Finally, do not forget to attend the typically Dutch Farewell Lunch Buffet at the end of the congress on Sunday afternoon. We are looking forward to your active participation in the 11th Congress.
### Abstract Book

**11th Congress of the European Hematology Association, Amsterdam, the Netherlands, June 15-18, 2006**

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Philadelphia chromosome positive leukemias
Myelodysplastic syndromes
Antibodies in the treatment of chronic lymphocytic leukemia
Clinical studies in non-Hodgkin’s lymphoma

Publication Only

Abstracts published only
Iron Diseases

0001
5 YEARS OF COMBINED CHELATION THERAPY: A RADICAL CHANGE IN BETA-THALASSAEMIA MAJOR PATIENT CONDITION
K.F. Farmaki
General Hospital of Corinth, CORINTH, Greece

Background. Transfusional iron overload in Thalassaemia major is fatal in the second decade of life unless treated appropriately. The ultimate goal of iron chelation therapy is to prevent organ damage and premature death. Combined chelation with Deferoxamine (Ferriprox®) & Desferrioxamine (Desferal®) produces a synergistic iron chelating effect that is difficult to achieve with either drug alone. This approach may place all patients in negative net iron balance and lead to a significant reduction of the body iron load. Aim. To show which iron-induced complications may be reversible with the use of combined chelation in Thalassaemia major patients. Methods. 50 β-Thalassaemia major patients (TMs) aged 6-46 years, switched from Desferrioxamine monotherapy to combined chelation with oral Deferoxprone (25-30 mg/kg t.i.d) and Desferrioxamine (20-50 mg/kg, 8-12 h SC or IV 2-6 days/week), in a 5 year regimen, adjusted on individual needs. The following tests were routinely performed: - mean annual Ferritin based on monthly measurements by MEIA; - annual/biannual ECG and Cardiac Echo for evaluation of cardiac function; - non-invasive heart & hepatic iron quantification, by annual endocrinology screening. Results. 1) None of the 50 TMps died since combined chelation treatment was implemented, while, with desferrioxamine monotherapy, mortality fluctuated from 15.5 to 14.5% over the last decade. 2) A trend analysis (PROC MIXED in SAS), revealed a negative trend of serum ferritin over time (p<0.0001) with a rate of decline equal to -95 ng/mL/month and a cumulative decrease in 5 years. In the 88.7% of compliant TMs the mean ferritin value at baseline (2.421 µg/L) decreased dramatically (107 µg/L) after 5 years of treatment. 3) In 12 patients with pre-existing heart dysfunction, symptoms (arrhythmias, hypertension and edema) reversed and heart medications were stopped. Ventricular dimensions and function normalized in Echo tests. Mean LVEF increased significantly (p<0.0001) from 54% to 72% following combined therapy. No case of new onset cardiac disease or worsening of pre-existing cardiac dysfunction was evident. 4) MRI measurements (T2 & T2* sequences) revealed significant reduction of iron overload in both organs over time leading to virtually iron free organs (Table 1).

Table 1.

<table>
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<tr>
<th>N=50</th>
<th>Mean T2H</th>
<th>Mean T2*H</th>
<th>Mean T1</th>
<th>Mean T1*</th>
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<tr>
<td>Normal values</td>
<td>&gt;35 mSec</td>
<td>&gt;28 mSec</td>
<td>&gt;33 mSec</td>
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</tr>
<tr>
<td>Desferal® monotherapy</td>
<td>28.2 mSec</td>
<td>22.7 mSec</td>
<td></td>
<td></td>
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<tr>
<td>After 3-Sys Combined Chelation</td>
<td>38.1 mSec</td>
<td>34.8 mSec</td>
<td>37.2 mSec</td>
<td>31.7 mSec</td>
</tr>
<tr>
<td>Difference</td>
<td>9.9 mSec</td>
<td>14.5 mSec</td>
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5) At baseline, 7 Tmps (13%, mean age 38.7 years) had Insulin-dependent Diabetes and 22 (44%, mean age 32.5 years) had Impaired Glucose Tolerance. Following addition of dextrepro, glucose metabolism improved. Insulin production increased and Insulin resistance reduced (Table 2). 6) Reversal of secondary amenorrhea and spontaneous ovulation in individual cases was validated by LH, FSH, E2/Progesterone, ovarian and uterine ultrasound. Conclusions. Combined chelation with Desferal® & Ferriprox® seems to be the treatment of choice because of increased efficacy in a minimally intrusive way. The obvious improvement of cardiac function with reversal of cardiac complications and the removal of myocardial iron, led to zero mortality. Not only was abnormal glucose tolerance reversed, but also the cumulative glucose response improved significantly with this regimen. The reversal of secondary hypogonadism and the hope of creating a family improved the quality of life of Thalassaemia patients considerably.

0002
PEARSON SYNDROME IN AN INFANT HETEROZYGOS FOR C282Y ALLELE OF HFE GENE
T. Papajik,1 K. Kefala-Agopoulopou,1 E. Roolides,2 E. Karatza,3 A. Lazandou1, E. Farmaki,1 P. Augoustides-Savvopoulou1, C. Tsantali2, J. Tsiousi1
1University Hospital, OLOMOUC, Czech Republic; 2Aristotle University, THESSALONIKI, Greece; 3Theagenion Hospital, THESSALONIKI, Greece

Background. Pearson syndrome is a rare mitochondrial disorder characterized by sideroblastic anemia that usually presents since infancy. Liver disease, renal tubulopathy and exocrine pancreas deficiency emerge later in the course of the disease. The syndrome is due to heteroplasmic mitochondrial DNA deletions and rearrangements, the lack of which, however, cannot exclude the disease. Diagnosis is made by clinical criteria and confirmed by genetic findings. Aim. To report the second case of Pearson syndrome in an infant heterozygous for C282Y allele of HFE gene. In addition, it is the first reported case successfully treated initially by deferoxamine and subsequently complicated by primary cutaneous zygomycosis. Case report. A 2-month old girl suffered from severe anemia since birth. Bone marrow examination revealed ring sideroblasts and signs indicative of dyserythropoiesis. Onset of anemia was accompanied by neutropenia that did not respond to administration of granulocyte colony-stimulating factor. M-FLSH (Fluorescent in situ hybridization) canotype was normal. There was neither deletion nor metathesis of any of the chromosomes. Metabolic evaluation was initially normal. Psychomotor development was normal, but the infant grew on the 9th percentile of weight and height. Transaminasemia developed when she was 8 month old, accompanied by thrombocytopenia. Transferrin saturation increased to 54% and ferritin reached the level of 3000 ng/dL. Ultrasonography revealed signs of diffuse-non specific damage of the liver. Deferoxamine was initiated and liver dysfunction subsided. Genetic evaluation revealed that the patient was heterozygous for C282Y allele of HFE gene. Pyridoxine and B12 per os were initiated but responded minimally. After 3-5 yrs, a 14-month old girl developed pneumonia due to Pneumocystis carinii lung infection evolved rapidly. Neurological disturbances not due to CNS infection developed. Multiple myeloma cutaneous zygomycosis. Liposomal amphotericin B was initiated while deferoxamine and corticosteroids were discontinued. Ultrasonography revealed diffuse liver damage had been reversed. Renal tubulopathy presented shortly before discontinuation of antifungal therapy. Enlargement of kidneys and liver was developed. Lactic acid increased (>500 mg/dL) and acidosis became severe. Acute Respiratory Distress Syndrome due to Pneumocystis carinii lung infection evolved rapidly. Neurological disturbances not due to CNS infection developed. Multiple myeloma cutaneous zygomycosis. Liposomal amphotericin B was initiated while deferroxamine and corticosteroids were discontinued. Ultrasonography revealed diffuse liver damage had been reversed. Renal tubulopathy presented shortly before discontinuation of antifungal therapy. Enlargement of kidneys and liver was developed. Lactic acid increased (>500 mg/dL) and acidosis became severe. Acute Respiratory Distress Syndrome due to Pneumocystis carinii lung infection evolved rapidly. Neurological disturbances not due to CNS infection developed.
organ dysfunction followed. The patient died at the age of 13 months.

Conclusion. Sideroblastic anemia in neonates is unusual and requires specific differential diagnosis. Metabolic disorders are among them and especially those of mitochondria. We report this case as a rare disease having uncommon complications: 1) Pearson syndrome emerged with hematological features until the age of 11 months. 2) Signs of evolution of the disease presented at a time when differentiation from anaphylactic shock was difficult. The infant was admitted to a level IV neonatal care intensive care unit. The patient was anemic, jaundiced, and required frequent transfusions. 3) Iron overload. 4) Deferoxamine therapy initially reversed liver damage. 5) High clinical suspicion is necessary for early recognition of rare infections (e.g. zygomycosis). Iron overload, hemochromatosis, deferoxamine and corticosteroids are underlying conditions for developing zygomycosis.

0003
CHELATION THERAPY WITH DEFERASIROX VERSUS DEFEROXAMINE IN TRANSFUSION-DEPENDENT SICKLE-CELL DISEASE: A COST-EFFECTIVENESS ANALYSIS FROM THE US PERSPECTIVE
E. Delea,1 O. Sofrygin,1 J.F. Baladi,2 S.K. Thomas,2 T.D. Coates3
1Policy Analysis Inc. (PAI), BROOKLINE, MA, USA; 2Novartis Pharmaceuticals Corporation, FLORHAM PARK, NJ, USA; 3Children’s Hospital of Los Angeles, LOS ANGELES, CA, USA

Background. Patients with sickle-cell disease (SCD) receiving chronic transfusions require chelation therapy to prevent complications of iron overload. Although deferoxamine is an effective iron chelator, it must be administered as an 8-12 hour infusion 5-7 times per week, leading to poor compliance and/or quality of life. Deferasirox is an once-daily oral iron chelator that produces reductions in liver iron concentrations and serum ferritin in 60% of patients and does not produce bone marrow suppression. To further evaluate from a US perspective the cost-effectiveness of deferasirox versus deferoxamine in SCD patients receiving frequent transfusions. Methods. Data from a variety of published and unpublished sources were used to estimate the cost-effectiveness of chelation therapy with deferasirox versus deferoxamine in SCD patients receiving frequent transfusions (3 per year). As there are no long-term studies describing the complications of iron overload in patients with SCD, we focused on the short-term (i.e., one year) costs and quality-of-life effects of chelation therapy. We assumed that patients would receive dosages of deferasirox and deferoxamine that have been found to be similar in patients with SCD (17.5 and 36.0 mg/kg/d respectively). To be conservative we assumed that all patients would be fully compliant with chelation therapy and that use of deferasirox therefore would have no effect on risk of complication of iron overload. Cost-effectiveness was measured in terms of the ratio of the difference (deferasirox vs deferoxamine) in mean incremental costs over a one year period to the gain in incremental mean QALYs over a one year period. Unit costs of deferoxamine and deferasirox were based on US wholesale acquisition costs. The cost of defereroxamine administration was based on analyses of health insurance claims data for US patients with transfusion-dependent anemias. Utilities (weights representing patient quality of life) were based on results of a study that used time-trade-off methods to estimate community-based preferences for oral versus infusional iron chelation therapy. Results. One year of treatment with deferasirox is estimated to result in a gain of 0.25 QALYs (0.82 vs 0.57 with deferoxamine). If the price of branded deferoxamine is employed, total annual costs were estimated to be $523 lower with deferasirox versus deferoxamine ($29,304 vs $29,827). Deferasirox therefore dominates deferoxamine (i.e., is less costly and results in more QALYs). If the price of generic deferoxamine is employed, the cost per QALY gained with deferasirox versus deferoxamine was $13,028. Cost-effectiveness of deferasirox vs deferoxamine was sensitive to the assumed dosages of deferoxamine and deferasirox and the costs and quality of life decrements associated with infusional therapy. Conclusion. In patients with SCD receiving frequent transfusions, deferasirox is less costly and yields more QALYs than branded deferoxamine. Compared with generic deferoxamine, the cost per QALY gained with deferasirox versus deferoxamine is well within the range that is generally considered acceptable in the US. Further research is needed to assess the potential implication of deferasirox on the risk-benefit profile of transfusion therapy in patients with SCD.

0004
MANNOSE BINDING LECTIN LEVELS IN THALASSEMIC PATIENTS WITH HEPATITIS C TREATED WITH PEGINTERFERON ALPHA-2A
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1Aghia Sophia Children’s Hospital, ATHENS, Greece; 2Athens University, ATHENS, Greece

Mannose-binding lectin (MBL) is a serum protein belonging to the family of collectins, which plays a critical role in the innate immune response. MBL is an acute-phase reactant of hepatic origin that can bind through multiple lectin domains to repeating mannose and N-acetylglucosamine sugar motifs that are characteristically displayed at high densities on bacterial and viral cells and on mammalian cells. After binding to a pathogen, MBL initiates at least 2 protective functions that are well defined. First, through the lectin pathway, MBL can mediate the activation of the complement system without the participation of antibodies; second, MBL can promote opsonophagocytosis by collecting receptors directly. Experiments in vitro and in vivo have shown that an MBL deficiency is likely to have a major effect on innate immune activation and appears to predispose individuals to serious infection. The amounts of MBL in human plasma are genetically determined. We studied the effect of treatment with pegylated interferon-α (peginterferon alpha-2a) on MBL levels in thalassemic patients with hepatitis C. Fourteen thalassemic patients with hepatitis C were included in the study. Eight with the hepatitis C genotypes 1 and 4 were treated for 48 wks, while 6 with the genotypes 2 and 3 were treated for 36 wks respectively, with subcutaneous infusions of peginterferon alpha-2a (Pegasys, Roche, Basel, Switzerland). MBL levels were measured by means of a fully auto-analyser and immunonephelometric assay on the BN-100 nephelometer (Dade Behring, Liederbach, Germany). The measurements were performed before and at the end of the treatment with peginterferon alpha-2a. MBL levels were increased significantly in 11/14 patients, independently from the therapeutic scheme, from 2,000±21 mg/L to 2,793±57 mg/L (p<0.108). In the three other patients the MBL levels remained unchanged and relatively low indicating a possible genetic influence. These findings suggest that administration of peginterferon alpha-2a in thalassemic patients with hepatitis C, additionally to the reduction of the observed viral load, normalizes the secretion of MBL and thus restore the impaired innate immunity system.

0005
EFFECTIVENESS AND SAFETY OF LONG-TERM COMBINATION IRON CHELATION THERAPY WITH DESFEROXAMINE & DEFERIPRONE IN MULTITRANSFUSED PATIENTS WITH THALASSEMIA
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Background. Transfusional haemochromatosis and increased dietary iron absorption cause severe complications in thalassaemic patients and lead to death before the 3rd decade of life unless treated effectively with iron chelation therapy. In this context, accurate assessment of body iron in these patients is essential for monitoring chelation in order to avoid toxic effects of iron overload and to prevent side effects from hyper doses of the chelator. Aim. The aim of this study is to evaluate the efficacy and safety of a combination therapy with the two chelators DFO and DEP, shown to have an additive or synergistic effect when used appropriately in severely loaded thalassaemic patients presenting with cardiac or liver complications. Methods. Twelve patients (5 men and 7 women; mean age 36, range 22 to 46 years) have been treated with DFO 40 mg/Kg/day 2 days/week and DEP 75 mg/kg/day (both chelators used on the same day). Four patients had a medical history of diabetes mellitus type II. Another four had been infected with HCV and two progressed to chronic active hepatitis. Iron load was estimated with serum ferritin and 24-hour urinary iron excretion (UIE) every 3 months. Cardiac and liver function and iron load were measured annually with biochemical tests (ALT, AST and γ-GT), ECHO cardiography and magnetic resonance imaging (MRI-T2). Results. Compliance with treatment was very high throughout the study period. No side effects or adverse reactions associated with combination therapy were observed. In patients presenting with cardiac dysfunction before treatment, symptoms disappeared two years after the onset of therapy. As shown in Table 1 serum ferritin decreased significantly (p=0.010), while UIE was significantly increased. As regards myocardium and liver, T2 relaxation time was significantly increased (p=0.002 and p=0.048 respectively), while no significant changes in liver enzymes were observed after treatment. Left Ventricular Ejection Fraction (LVEF) (p=0.007) and fractional shortening were significantly increased.
The results of the secondary endpoint revealed both therapies were associated with a significant reduction in LIC (−2.4±3.2 and −1.4±4.0 for deferasirox and deferiprone respectively; p<0.001 for both). The overall reduction was due mainly to the effect of the chelators on the LIC of patients with greater baseline LIC. Conclusions. The overall success for the primary efficacy endpoint was greater for deferiprone than deferasirox. The success of the highest doses of deferasirox was similar to that of deferiprone at 75 100 mg/kg/day. Although these data were generated from distinct cohorts of patients participating in independent studies, they represent carefully conducted studies in the same types of patients and provide a means for obtaining an initial comparison. These results highlight the need for a randomized study comparing the two chelators, and one where only effective doses of deferasirox will be used.

## Table 1

<table>
<thead>
<tr>
<th>LIC at baseline</th>
<th>Exjade™ primary efficacy success criteria</th>
<th>Ferritron™ primary efficacy success criteria</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-&lt;7 mg Fe/g dw</td>
<td>34/85 (84%)</td>
<td>22/27 (81%)</td>
<td>0.0002</td>
</tr>
<tr>
<td>≥7 mg Fe/g dw</td>
<td>112/191 (59%)</td>
<td>20/33 (61%)</td>
<td>0.83</td>
</tr>
<tr>
<td>Overall</td>
<td>146/276 (53%)</td>
<td>42/60 (70%)</td>
<td>0.016</td>
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</table>

Conclusions. Our results show high acceptance of long-term combination therapy with DFO and DFP by patients who previously failed to comply with DFO monotherapy. Long-term administration of both chelators used on the same day has been shown to be safe and no deleterious effects were observed. Serum ferritin was positively correlated with cardiac ECHO and MRI. No noteworthy change was found in liver iron, possibly due to the late onset of chelation and consequent permanent liver damage.

## 0007

**THE ROLE OF RETICULOCYTE HEMOGLOBIN CONTENT AS IRON STATUS MARKER IN HYPOPLASIA PATIENTS**

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**Background.** Iron deficiency leads the hyporesponsiveness to erythropoietin (rHuEPO) in hemodialysis patients and results in renal anemia. So, the early detection of iron deficiency is of value for the successful treatment of renal anemia. At present, serum ferritin and transferrin saturation (TS) are recommended for assessing iron deficiency. However, they have a limitation in estimating iron status because the lack of accuracy and precision in dialysis patients. The reticulocyte hemoglobin content (CHr) has been proposed as a useful tool in iron status assessment, but its cutoff value for iron deficiency varies from 26 to 32 pg in different studies. **Aims.** We investigate the accuracy of CHr in comparison to the conventional test and a CHr cutoff value. Also, we assess that the CHr change after administration of iron supplement related to changes in red cell count, Hb, and Hct. **Methods.** We selected 163 hemodialysis patients (95 females and 78 males, mean age 56.1±13.2) receiving rHuEPO and oral or intravenous iron therapy. We measured CBC, reticulocyte, CHr (using ADVIA120 autoanalyzer, Bayer Medical, USA), iron parameters (iron, TIBC, ferritin), CRF, BUN and creatinine. Iron deficiency in this study was defined as a serum ferritin < 100 µL or a TS < 20%. In patients categorized as iron deficient, CBC, reticulocyte, and CHr were determined at 1 month after iron therapy. **Results.** The mean Hb in hemodialysis patient was 10±1.1 g/dL and 53 patients were iron deficient (19 with low ferritin and low TS, 8 with only low ferritin, 26 with only low TS). CHr were distributed with mean 35±1.4 pg in iron sufficient group and mean CHr 29±2±1.2 pg in iron deficient group, and showed significant difference between 2 groups. CHr was positively correlated with TS (r=0.36, p=0.02), but there was no correlation with iron, ferritin, BUN and creatinine. The CHr changes were related to changes in red cell count (r=0.13, p=0.045) and Hct (r=0.21, p=0.05). **Conclusions.** CHr is available in measuring iron status in dialysis patients, especially in patients with iron deficiency, but its cutoff value for iron deficiency varies from 26 to 32 pg is appropriate for the assessment of iron deficiency (sensitivity 100%, specificity 80%). Also, CHr might be useful to predict the degree of erythropoietic response after iron administration.

## 0008

**SENSITIVITY ANALYSIS ON THE COST-EFFECTIVENESS OF CHELATION THERAPY WITH DEFERASIROX OR DEFEROXAMINE IN TRANSFUSION-DEPENDENT THALASSEMAIA PATIENTS BASED ON EUROPEAN COSTS**

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**Background.** Deferoxamine is an effective iron chelator but must be administered as an 8-12 hour infusion 5-7 times per week, leading to poor compliance, effectiveness, and/or quality of life. Deferasirox is a novel once-daily oral chelator that produces reductions in liver iron concentrations (LIC) and serum ferritin similar to deferoxamine, and has been found to have a favourable cost effectiveness in US thalassemics. Cost-effectiveness in other settings has not been examined. **Aims.** To examine the sensitivity of the cost-effectiveness of deferasirox and deferoxamine among thalassemia patients to costs prevailing in various European countries. **Methods.** A Markov model developed previously for the

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US was adapted to examine the potential cost-effectiveness of deferasirox and deferoxamine in various European countries, using ranges of values for costs of chelation which may prevail across these settings. Other inputs were unchanged as they are likely to be similar across settings. Patients were assumed to have thalassemia major, be three years of age at initiation of chelation therapy, and to receive prescribed dosages of deferasirox and deferoxamine that have been shown to be safe and efficacious in patients with LIC >27 Fe/g dry weight (24.6 and 47.2 mg/kg/d respectively). Compliance with deferoxamine was based on analyses of health insurance claims data. Because data on compliance with deferasirox versus deferoxamine are unavailable, published data on compliance with the oral chelator deferiprone versus deferoxamine were used. Probabilities of complications of iron overload and death by clinical factors, satisfaction with ICT, and side effects were estimated from published studies. Differences in quality of life with deferasirox versus deferoxamine were based on a study of patient preferences for oral versus intravenous chelation therapy. The price of deferoxamine was varied from €15 to €40 per 2 g vial; the price of deferasirox, from €40 to €50 per 1 g vial; and the cost of deferoxamine administration, from €10 to €40 per infusion. Costs of complications of iron overload conservatively were not considered. Cost-effectiveness was defined as the incremental cost per quality-adjusted life years (QALY) gained. Future costs and QALYs were discounted at 5% annually.

Results. Compared with no chelation (which yields 7.6 QALYs), deferoxamine yields an additional 4.1 QALYs per patient while deferasirox yields an additional 8.1 QALYs per patient. Expected lifetime costs of chelation therapy with deferoxamine range from €70,000 to €226,000 per patient; those for deferasirox range from €186,000 to €266,000 per patient. Cost-effectiveness versus no chelation ranges from €20,000 to €63,000 per QALY gained for deferoxamine and from €28,000 to €35,000 per QALY gained for deferasirox. In almost all scenarios where the cost of deferoxamine administration is €15 per infusion or more, the cost-effectiveness of deferasirox versus no chelation is more favorable than that of deferoxamine versus no chelation. The cost-effectiveness of deferasirox versus deferoxamine was less than that of iron overload (€40,000 per patient) in almost all scenarios. Conclusion. Although analyses based on actual prices of deferasirox are necessary, this analysis suggests that the cost-effectiveness of deferasirox versus deferoxamine or no chelation in European settings is within the range considered acceptable in these countries.

0009
BURDEN OF IRON CHELATION THERAPY SIGNIFICANTLY IMPACTS ADHERENCE TO TREATMENT IN PATIENTS WITH IRON OVERLOAD
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Background. As part of a supportive care programme, thalassemia, sickle cell disease (SCD), and myelodysplastic syndrome (MDS) patients require regular blood transfusions. One consequence of this is iron overload and, more importantly, earlier mortality. Current iron chelation therapy (ICT) using deferoxamine (DFO) requires 8-12 hour infusions, 5-7 days per week. This potentially limits health related quality of life and inhibits adherence in patients with thalassemia, SCD, and MDS. Aims. To assess adherence to ICT amongst patients with thalassemia, SCD, and MDS, and to investigate the extent to which patient adherence with ICT is attributable to socio-demographic and clinical factors, satisfaction, and self esteem factors. Methods. Patients with thalassemia, SCD, or MDS currently undergoing ICT completed a 28-item satisfaction with ICT questionnaire comprised of four domains, namely: Perceived Effectiveness, Burden, Acceptability, and Side Effects, as well as three items on adherence to ICT. We analysed data from 110 patients, and performed simple linear regression analyses followed by multivariate regression analysis (with backward selection process) to assess the joint effects of age, whether patients experience side effects, satisfaction with ICT (perceived effectiveness; burden on ICT, acceptability of treatment, and side effects), and self esteem (feelings about yourself). Results. The mean age was 30.87 years (SD=14.95). Overall, patients who experienced side effects in the previous 30 days were significantly more likely to think about stopping their ICT than those who did not experience side effects (p=0.02). Six variables were identified in the univariate analysis as significant predictors of thinking about stopping medication. Satisfaction with ICT independently explained the most variance and was positively associated with never thinking about stopping medication (higher satisfaction scores were related to never thinking about stopping medication), followed by satisfaction with side effects, acceptability of ICT, age, perceived effectiveness, and then feelings about yourself (Table: Simple regression analyses (R2, p values)). The multivariate regression model explained 42.3% of the total variance of thinking about stopping medication. Specifically, a significant positive relationship was demonstrated between never thinking about stopping medication and age (p=0.04), perceived effectiveness (p=0.0005), burden on ICT (p=0.002), and satisfaction with side effects (p=0.01). Summary/Conclusions. This study proposes a framework to understand the complex set of factors associated with adherence to ICT. This analysis shows the following determinants of adherence in order of importance: satisfaction with burden of ICT, satisfaction with side effects, satisfaction with acceptability of ICT, age, perceived effectiveness, and feelings about yourself. These aspects should be considered as part of any chelation care programme. More effective, more convenient ICT will lead to greater satisfaction with therapy and reduce the likelihood of reductions in compliance. Further research is necessary to obtain greater certainty about these relationships and the direction of the relationships.

<table>
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<th>Table 1.</th>
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<tr>
<td>Significant predictors of thinking about stopping medication</td>
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<tr>
<td>Satisfaction with burden</td>
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<tr>
<td>Satisfaction with side effects</td>
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<tr>
<td>Acceptability</td>
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<td>Age</td>
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<tr>
<td>Perceived effectiveness</td>
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<td>Feelings about yourself</td>
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0010
EFFECTS OF SILYMARIN ON TELOMERE ACTIVITY AND PROLIFERATION OF PERIPHERAL BLOOD T-LYMPHOCYTES IN THALASSEMIA MAJOR PATIENTS?
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Background. Iron, an essential growth trace element, is required for proliferation of all living cells, including T lymphocytes. Many, though not all, immune responses require lymphocyte proliferation. But iron overload, due to dyserythropoiesis and regular blood transfusion in β-thalassemia major patients, a major problem in Mediterranean as well as in Iran, is associated with impaired lymphocyte proliferative responses to mitogens and cell-mediated immunity. Iron mainly in its non-ferrous form, low molecular weight form, cause cellular damage by participating in the generation of the hydroxyl radical, thought to be the principal effector of oxidative DNA damage. One possibility is that telomerase activity, essential enzyme for the repair of telomeric DNA, is reduced following damage by oxygen radicals. Aims. The aim of the present study was to investigate telomerase activity in lymphocytes from patients with iron overload disease and to observe its regulation of cellular proliferation and also evaluate effect of Silymarin on this enzyme. Methods. Peripheral blood mononuclear cells (PBMC) were isolated from 20 patients with β-thalassemia major and 20 healthy donors. Cells were stimulated with PHA and treated with Deferoxamine and Silymarin for 72 h. Telomerase activity was measured by the telomeric repeat amplification protocol (TRAP) based telomerase polymerase chain reaction enzyme linked immunosorbent assay. DNA synthesis of the cells was assayed using BrdU (5-bromo-2'-deoxyuridine) incorporation. Results. The results showed that telomerase activity of resting peripheral lymphocytes of healthy subjects and patients with β-thalassemia major was detectable at low level, and obviously increased after stimulation in vitro with phytohaemagglutinin and PHA following iron overload treatment. There is no influence of treatment with Deferoxamine (DFO). The decreased telomerase activity of resting lymphocytes was found in patients with β-thalassemia major compared to that in healthy subjects. The DNA proliferation was paralleled by increase in telomerase activity. Conclusions. These results leads to important conclusions. First, the ability of T cells to upregulate Telomerase activity upon activation may decrease over time (aging) and following iron overload-mediated oxidative stress. Second, Silymarin upregulates telomerase activity of T-Lymphocytes and Deferoxamine downregulates. Third There is a direct correlation between telomerase...
activity and cell proliferation. One possibility is that telomerase is essential for the repair of telomeric DNA following damage by oxygen radicals. Finally, because telomerase contributes to protection from telomere shortening in activated lymphocytes, it may play a critical role in immune responses and also Silymarin contributes as a Superantioxidant may strengthen immune function through scavenging free radicals and upregulation of protective enzymes. In addition, waves of proliferation followed by extensive cell death, the limitation in cell division imposed by oxidative stress-mediated reduced telomerase activity and Telomere shortening may contributes immunodeficiency.

**0011**

HEPCIDIN MUTATION IN A BETA-THALASSEMIA MAJOR PATIENT WITH PERSISTENT SEVERE IRON OVERLOAD DESPITE CHELATION THERAPY

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University of Milan-Policlinico Hospital, MILAN, Italy; University of Messina-C. Marini Hospital, MESSINA, Italy

**Background.** Hepcidin is a peptide hormone produced in the liver; it is an important negative regulator of iron absorption from the enterocytes and of iron release from macrophages. Hepcidin dysregulation is implicated in the pathogenesis of several iron disorders. Iron overload and inflammation up-regulate hepcidin synthesis decreasing dietary iron absorption, while anaemia and hypoxia suppress hepcidin expression. Thalassemia Major (TM) is a hereditary haemolytic anaemia requiring long-life blood transfusions treatment. Iron storage in patients undergoing regular transfusion is responsible for the impairment of heart, liver and hence reduced survival. Iron chelation treatment is required to reduce the morbidity and mortality associated with iron overload secondary to chronic transfusion therapy. Aim. Despite regular iron chelation some thalassemia patients have persistent high ferritin levels. To get further insights in this issue, several factors have been investigated including infections, inflammatory status and coexistence of HFE and hepcidin mutations. Patients and Methods. We report a case of TM patient with severe iron overload despite regular chelation therapy, carrying mutations in hepcidin and HFE genes. The proband is a 23-years-old homozygous b039 woman, regularly transfused since the age of 1 year and had hypogonadism and hypothyroidism. Serum ferritin levels (Cr) or clearance of creatinine (Ccr) is often misleading. The early development of glomerular hypertrophy enhances creatinine excretion and gives false normal results of both Cr and Ccr. Therefore, the renal dysfunction becomes evident rather late. For that reason, the identification of markers that indicate early renal dysfunction as well as further progression to end-stage renal disease is highly desirable. Cystatin C (Cys-C) is a cysteine protease inhibitor, which serves as an endogenous parameter of GFR, while β2-microglobulin (β2-M) is a sensitive marker of the glomerular filtration capacity of the kidney. Finally, N-acetyl-β-D-glucosaminidase (NAG), a widely distributed lysosomal enzyme found predominantly within the renal proximal tubules is also a sensitive indicator of renal injury. Aim. The aim of this study was to evaluate whether Cys-C, β2-M and NAG excretion may serve as early indicators of renal dysfunction in a large cohort of HbS/β-thal patients. To our knowledge, such studies are not available in the literature. Patients and Methods. We studied β7 compound, HbS/β-thal patients (56M/51F; median age 39 years) and 30 healthy controls. All patients were Caucasians, of Greek origin, had stable disease at the time of evaluation, without sickle-cell crises or infections, and had not been transfused for at least three months before. Serum Cys-C and β2-M were determined by particle enzyme immunoassay and performed using the Dade Behring BN Prospec nephelometer. Urine NAG activity was measured by a colorimetric assay (Roche Diagnostics, Mannheim, Germany) and expressed as daily output in U/day. Results. Cys-C, NAG and serum β2-M levels were higher in patients than controls (p<0.01, <0.0001, and <0.0001, respectively). The incidence of patients with high levels of Cys-C, NAG and β2-M was 32.1%, 74.7% and 70.1% respectively, while only 6.2% of patients had increased serum creatinine levels. Cys-C and serum β2-M showed a strong correlation with Ccr (r=0.48, p<0.0001; and r=0.38, p<0.001, respectively), while NAG positively correlated with proteinuria (r=0.546, p<0.0001). An inverse strong correlation was also observed between hemoglobin and β2-M (r=0.53, p<0.004), NAG and Cys-C levels (r=0.315, p<0.002). Seven patients with proteinuria received therapy with ACE-inhibitors. Changes of proteinuria positively correlated with NAG levels (r=0.691, p<0.001). Conclusions. These results indicate that Cys-C is an accurate marker of renal dysfunction, and urinary NAG excretion can be considered as a reliable index of the tubular toxicity, and possible predictor of proteinuria and eventual renal impairment in HbS/β-thal patients. Furthermore, NAG measurement may be used for monitoring ACE-inhibitors therapy in HbS/β-thal patients with proteinuria.

**0012**

**EARLY MARKERS OF RENAL DYSFUNCTION IN PATIENTS WITH SICKLE CELL/β-THALASSEMIA**

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**Background.** Progressive renal failure is one of the main complications in sickle cell/β-thalassemia (HbS/β-thal). Detection of the progressive renal damage using conventional parameters, such as serum creatinine levels (Cr) or clearance of creatinine (Ccr) is often misleading. The early development of glomerular hypertrophy enhances creatinine excretion and gives false normal results of both Cr and Ccr. Therefore, the renal dysfunction becomes evident rather late. For that reason, the identification of markers that indicate early renal dysfunction as well as further progression to end-stage renal disease is highly desirable. Cystatin C (Cys-C) is a cysteine protease inhibitor, which serves as an endogenous parameter of GFR, while β2-Microglobulin (β2-M) is a sensitive marker of the glomerular filtration capacity of the kidney. Finally, N-acetyl-β-D-glucosaminidase (NAG), a widely distributed lysosomal enzyme found predominantly within the renal proximal tubules is also a sensitive indicator of renal injury. Aim. The aim of this study was to evaluate whether Cys-C, β2-M and NAG excretion may serve as early indicators of renal dysfunction in a large cohort of HbS/β-thal patients. To our knowledge, such studies are not available in the literature. Patients and Methods. We studied 87 compound, HbS/β-thal patients (56M/51F; median age 39 years) and 30 healthy controls. All patients were Caucasians, of Greek origin, had stable disease at the time of evaluation, without sickle-cell crises or infections, and had not been transfused for at least three months before. Serum Cys-C and β2-M were determined by particle enzyme immunoassay and performed using the Dade Behring BN Prospec nephelometer. Urine NAG activity was measured photometrically at 580 nm using a colorimetric assay (Roche Diagnostics, Mannheim, Germany) and expressed as daily output in U/day. Results. Cys-C, NAG and serum β2-M levels were higher in patients than controls (p<0.01, <0.0001, and <0.0001, respectively). The incidence of patients with high levels of Cys-C, NAG and β2-M was 32.1%, 74.7% and 70.1% respectively, while only 6.2% of patients had increased serum creatinine levels. Cys-C and serum β2-M showed a strong correlation with Ccr (r=0.48, p<0.0001; and r=0.38, p<0.001, respectively), while NAG positively correlated with proteinuria (r=0.546, p<0.0001). An inverse strong correlation was also observed between hemoglobin and β2-M (r=0.53, p<0.004), NAG and Cys-C levels (r=0.315, p<0.002). Seven patients with proteinuria received therapy with ACE-inhibitors. Changes of proteinuria positively correlated with NAG levels (r=0.691, p<0.001). Conclusions. These results indicate that Cys-C is an accurate marker of renal dysfunction, and urinary NAG excretion can be considered as a reliable index of the tubular toxicity, and possible predictor of proteinuria and eventual renal impairment in HbS/β-thal patients. Furthermore, NAG measurement may be used for monitoring ACE-inhibitors therapy in HbS/β-thal patients with proteinuria.

Anemia/Red blood cells I

**0013**

A PHASE I, SINGLE AND FRACTIONATED, ASCENDING DOSE STUDY EVALUATING THE SAFETY, PHARMACOKINETICS, PHARMACODYNAMICS, AND IMMUNOGENICITY OF AN ERYTHROPOIETIC MIMETIC ANTIBODY FUSION PROTEIN, CTN0528, IN HEALTHY MALE SUBJECTS


CHDR, LEIDEN, Netherlands; Centocor, Inc., MALVERN, USA

**Objectives.** To assess the safety, pharmacokinetics (PK), pharmacodynamics and immunogenicity of single and fractionated IV doses of an erythropoietic mimetic antibody fusion protein, CTN0528, in healthy male subjects.
males. Methods: In this randomized, single blind, and placebo (PBO)-controlled study, 57 subjects were enrolled in 5 dose cohorts. In Stage 1, 35 subjects received a single IV administration of 0.03, 0.09, 0.3, 0.9 mg/kg CNTO 528 or PBO. In Stage 2, 9 subjects received fractionated IV administrations of CNTO 528 or PBO on Days 1, 3 and 5 (3 infusions of 0.09 mg/kg or PBO). Results. Pharmacodynamics: In subjects treated with IV CNTO 528, a dose dependent increase in reticulocyte counts was observed. A dose dependent increase in RBC count was observed with all RBC indices (MCV, MCH, MCHC) with a normal range, indicating an increase in normocytic, normochromic RBCs. In all CNTO 528 treated subjects, a dose-dependent increase in soluble transferrin receptor concentration was observed. A dose-dependent increase in endogenous EPO concentration was observed, followed by a dose dependent decrease in endogenous EPO concentration. Pharmacokinetics: In the single dose part of the study, Cmax and AUC increased in an approximately dose proportional manner. The mean terminal half-life ranged between 6 - 7 days in the higher dose cohorts. Safety: Treatment with CNTO 528 was generally well tolerated. There were no serious adverse events (AEs) and few CNTO 528-related AEs. Two subjects in the IV CNTO 528 cohort met the protocol pre-specified interruption rule of Hgb > 17.5 g/dL and underwent phlebotomy. In these subjects, high Hgb concentrations were not associated with AEs or clinical symptoms. All AEs were determined by the investigator to be mild to moderate in intensity. The most common AE across all groups was headache, occurring in both CNTO 528- and PBO-treated subjects. There was no dose-related trend across groups, and most subjects who experienced headaches were in the lowest 2 dose groups. There was no indication that any patterns of AEs or significant safety laboratory, vital signs, or ECG abnormalities were associated with the administration of CNTO 528. Immunogenicity: None of the 24 subjects who received single IV administration of CNTO 528 were positive for antibodies to CNTO 528. Conclusions. Single and fractionated IV administrations of CNTO 528 were well tolerated and resulted in prolonged, dose-dependent erythropoietic responses with notably low inter-subject variability. PK of IV CNTO 528 was linear and approximately dose proportional. This data provides the first proof of concept in humans for erythropoietic responses and an increase of endogenous EPO levels by an erythropoietic mimetic antibody fusion protein.

0014
CORRECTION OF ANEMIA OF THE POST-OPERATIVE PERIOD AFTER ORTHOPEDIC SURGERY BY ORAL VERSUS INTRAVENOUS IRON VERSUS INTRAVENOUS IRON + EPO: A PROSPECTIVE RANDOMIZED TRIAL
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Background. Approximately 20% of patients after orthopedic surgery (hip or knee replacement) present moderate to severe anemia (Hb between 75 and 105 g/L). They are often treated by oral iron for up to three months. Aims. We performed a prospective randomized pilot study to investigate the potential of iv iron or iv iron + EPO to treat this kind of anemia as compared to oral iron therapy. Methods. Of the 57 patients included in the study, 47 completed the trial and received either 80 mg of oral iron /day (Tardysteron®) for three months (19 patients), or 200 mg iv iron sucrose (Venoferr®) at post-operative-days (POD) 1, 3, 5 and 8 (13 patients), or 200 mg iv iron sucrose (Venoferr®) on the same PODs plus 150 IU/kg of EPO (epoietin alpha, EPREX®) on PODs 1, 3 and 5 (15 patients). Nadir post-operative mean Hb values were 141.3, 138.0, and 134.1 g/L, respectively. Nadir post-operative mean Hb values were 98 (POD 10), 91 (POD 8), and 94.5 (POD 8) g/L for the three groups, respectively. At POD 20, Hb (compared to nadir) was 12.5 g/L in the oral iron group, 15.5 g/L in the iv iron group, and 25.5 g/L in the iv iron + EPO group (p<0.016 group 3 versus groups 1 & 2). At POD 30, Hb was 27.5, 29, and 32.5 g/L, respectively, and 45.3, 48, and 42.5 g/L at POD 90. At day +30, 57%, 46%, and 60% of patients had normalized their Hb value (p=0.0266 group 3 versus groups 1 & 2). The CRP mean value at POD 1, 8, and 10 of 117, 56, and 34 mg/L respectively. Finally, ferritin levels at POD 90 were -29, +75, and +84 ug/L respectively. This pilot study clearly shows that in moderate to severe post orthopedic surgery anemia, the highest and most rapid increase in Hb was seen in the group of patients treated by iv iron + EPO. This difference is, in our opinion, due to the acute inflammatory state which developed secondary to surgery and lasted for almost 15 days. The study also shows that therapy with iv iron is well tolerated and, unlike oral iron therapy, allows a complete restoration of iron stores. The impact of this accelerated Hb recovery on quality of life, hospital stay duration and incidence of post-operative complications should be studied in a future trial with a larger patient population.
0016

BODY IRON BALANCE AND IRON EXCRETION: INTAKE RATIO, ACCORDING TO TRANSFUSIONAL REQUIREMENTS, DURING TREATMENT WITH THE ONCE-DAILY ORAL IRON CHELATOR DEFERASIROX (EXJAYE, ICL670)

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Background. In chronically transfused patients it is important to understand how much iron is removed by chelation therapy for a given rate of iron intake, to allow tailoring of treatment regimens to achieve the desired iron balance, i.e. maintenance in a well-controlled patient or reduction in an iron-overloaded patient. Deferasirox (Exjade®, ICL670) is a novel, once-daily oral iron chelator that was recently approved for the treatment of chronic transfusional iron overload in adult and paediatric patients aged ≥ 2 years. The efficacy and safety of deferasirox have been established in patients with a range of transfusion-dependent anaemias. Importantly, the efficiency of deferasirox is similar across a wide dose range (~28% over 5-30 mg/kg/day), indicating that the higher the dose, the more iron will be removed from the body. Aims. The aim of this post-hoc analysis, which pooled data from four pivotal deferasirox clinical trials, was to evaluate deferasirox in relation to change in net body iron (excretion) and the impact of transfusional requirements (intake) and to determine the iron excretion:intake ratio. Methods. A total of 1,005 patients (deferasirox n=652, deferoxamine [DFO, Desferal®] n=353) were stratified according to their transfusional requirements (0-2, 3-14, ≥ 15), and to determine the iron excretion:intake ratio. Results. Among the pooled population of deferasirox-treated patients, most (n=419, 64.3%) had intermediate transfusional requirements. When evaluating mean net iron balance and iron excretion:intake ratio in completing patients with baseline and end-of-study liver iron concentration (LIC) assessments and recorded positive iron intake (approximately 90% of overall population), a transfusion- and dose-related response pattern was observed with both deferasirox (n=556) and DFO (n=525). Mean net iron balance results for 10, 20 and 30 mg/kg/day doses are presented in Figure 1.

Figure 1. Mean net iron balance (g/year) by treatment, dose and transfusional requirements.

The mean iron excretion:intake ratio was less than 1 (intake exceeded excretion) in all patients receiving deferasirox 5 mg/kg/day, irrespective of transfusional requirements (0.52, 0.67 and 0.34 in the low, intermediate and high cohorts, respectively). Conclusions. Based on this analysis, deferasirox 10 mg/kg/day maintained iron balance in patients with low transfusional requirements, 20 mg/kg/day maintained or reduced iron balance in patients with low and intermediate requirements, while 30 mg/kg/day decreased iron balance in most patients, irrespective of transfusional requirements. Since deferasirox efficiency does not vary across doses, it is now known that 5 mg/kg/day is insufficient to maintain or reduce iron balance relative to patients’ transfusional requirements. Comparable effects were observed between DFO and deferasirox doses in a 2:1 ratio, indicating that an effective deferasirox dose will be around half that of an effective DFO dose. Deferasirox dosing should therefore be guided by transfusional requirements, severity of iron overload and treatment goal. In addition, as regular transfusions lead to iron accumulation, it is important to monitor transfusion rates, serum ferritin levels and/or LIC.

0017

EPOETIN BETA 30 000 IU ONCE WEEKLY IS EFFECTIVE AND WELL TOLERATED IN ANEMIC PATIENTS WITH SOLID OR NON-MYELOID HEMATOLOGICAL MALIGNANCIES RECEIVING CHEMOTHERAPY: RESULTS FROM THE NAUTICA STUDY

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Background. Anemia is a frequent complication of cancer, affecting about two-thirds of patients at some time during their illness (Ludwig et al., Eur J Cancer 2004). The symptoms of anemia have a profound impact on quality of life (QoL). Low hemoglobin (Hb) levels have also been associated with reduced tumor control and reduced survival. Epoetin β (Neorecombin®) is an effective treatment for anemia and is equally effective whether administered three times weekly or once weekly (QW) in patients with lymphoproliferative malignancies (Cazzola et al., Br J Haematol. 2005). Few studies, however, have evaluated this QW regimen in patients with a wider range of malignancies. Aims. To evaluate the efficacy and safety of epoetin β 30 000 IU QW in patients with solid or non-myeloid hematological malignancies. Methods. This was an open-label, single-arm study carried out in 87 centers throughout France between 31 December 2003 and 30 August 2005. Adult patients with solid or non-myeloid hematological malignancies and anemia (Hb levels < 12 g/dL), a WHO performance status 0-2 and who were scheduled to receive chemotherapy were enrolled. Patients received epoetin β 30 000 IU subcutaneously QW over 16 weeks. Follow-up visits were scheduled after each chemotherapy cycle. The primary efficacy parameter was change in Hb level during epoetin β therapy. Hb response was defined according to patients’ baseline Hb level. For patients with Hb levels of 11-12 g/dL at baseline, response was defined as achievement of Hb level of ≥13 g/dL, and, for those with Hb levels of <11 g/dL, response was defined as Hb increase of ≥2 g/dL. Results. A total of 691 patients were included in the intention-to-treat population. Mean age was 62.6 (SD, 13.1) years. Fifty-three percent of patients had solid tumors and 47% had hematological malignancies. The mean Hb level at baseline was 10.1 (SD, 1.1) g/dL. The mean Hb level at study endpoint was 12.0 (SD, 2.2) g/dL. The median duration of treatment was 14 weeks. Hb response was observed in 60% of patients during the study and the median time to response was 49 (range 10-130) days. Hb response was seen equally in patients with either hematological malignancies (60%) or solid tumors (61%). Likewise, Hb response was seen with all types of chemotherapy. The subgroup of patients with Hb <11 g/dL at entry, which corresponds to the intervention level provided in the current label for epoetin β. Conclusion. In patients with either solid tumors or non-myeloid hematological malignancies, epoetin β 30 000 IU QW effectively and rapidly increased Hb to target levels and was well tolerated.
PATIENT CONTROLLED ANALGESIA VERSUS CONTINUOUS INFUSION OF MORPHINE DURING VASO-OCCULSIVE CRISIS IN SICKLE CELL DISEASE: A RANDOMIZED CONTROLLED TRIAL

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Background. Pain during vaso-occlusive crisis (VOC) in sickle cell disease (SCD) is commonly treated with continuous intravenous infusion (CI) of morphine. During CI the treating physician titrates the dose of morphine until adequate relief of pain has been established. Patient controlled analgesia (PCA) allows the patient to self-administer doses of morphine for the relief of pain and has shown to be equalanalgic in post surgical patients with lower morphine consumption than with the CI of morphine. Morphine has many dose-related side-effects and high plasma levels of morphine are associated with serious complications. Aim. To compare the administration of morphine with PCA versus CI in sickle cell patients with VOC we conducted the first randomized controlled trial in this setting. Methods. Patients were randomized between PCA and CI of morphine within 24 hours after hospital admission. Endpoints of the study were: the mean and cumulative morphine dose, pain intensity and quality of life (QoL). Pain intensity was measured daily using a ten-point-scale verbal pain score. Reduction of pain intensity was measured by subtracting a pain score on a ten point visual analogue scale (VAS) before randomization from the same measurement two days after randomization. QoL was measured using the Medical Outcomes Study 36-item Short Form Healthy Survey (SF36). Results. Twenty-five consecutive episodes of VOC in 19 patients with SCD were included. Patients with PCA demonstrated to have significantly lower morphine consumption as compared to patients randomized to CI. The mean and total cumulative morphine dose was 0.5 mg/h and 33 mg in the PCA-group versus 2.1 mg/h and 275 mg in the CI-group, respectively (p<0.001 and p<0.001). In addition, a non-significant reduction in median duration of hospitalisation was found (6 versus 10). Despite the markedly reduced cumulative dose of morphine in the patients treated with PCA, no difference in pain intensity was found between the groups. The mean daily ten-point-scale verbal pain score was 4.9 in the PCA group versus 5.3, in the CI-group (NS). Also no difference in QoL was found. Conclusion. We conclude that the use of PCA in sickle cell patients with VOC results in adequate pain relief at a significant lower morphine dose as compared to morphine administration by continuous infusion.

EX Vivo ANALYSIS OF PKLR MUTATIONS THAT AFFECT CORRECT PROCESSING OF PKLR mRNA CAUSING PYRUVATE KINASE DEFICIENCY

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Background. Red blood cell pyruvate kinase (PK) deficiency is the most common cause of nonspherocytic hemolytic anemia due to defective glycolysis. The clinical picture varies from severe hemolysis causing neonatal death to a well compensated hemolytic anemia. PK deficiency is inherited in an autosomal recessive manner and caused by mutations in the PKLR gene. Most of these are missense mutations affecting conserved residues in structurally and functionally important domains of the protein. More rarely, PK deficiency is caused by mutations that lead to aberrant processing of PKLR pre-mRNA. Aims. We aimed to study the effect of mutations associated with PK-deficiency and postulated to affect PKLR pre-mRNA processing. Methods. Pro-erythroblasts were cultured ex vivo from patient-derived CD34+ cells and used as a source of erythroid-specific RNA. We used RT-PCR with fluorescent-dye-labeled primers, fragment analysis, cloning, and DNA sequence analysis of the clones to identify and characterize PKLR transcripts. Results. Five different mutations were studied. Two were located at the 5' splice site of exons 3 and 8 (c.283G>C-A and intron 11 (c.1618_1619+1delG)). Two mutations were located at the 5' splice site of intron 4 (IVS4-2A>C) and intron 11 (IVS11-3C>G). The fifth mutation was located in exon 8 (c.990C>T). The missense mutation c.283G>C in exon 3 encodes the substitution of glycine by arginine at residue 95. More importantly, this mutation altered the 5' splice site of IVS3. As a result most transcripts did not contain exon 3, coding for a PK monomer that, if translated, lacks amino acids 34 to 94. Similarly, the one bp deletion at the exon/intron boundary of IVS11, c.1618_1619+1delG, altered the 5' splice site of IVS11. This caused skipping of exon 11 in the majority of transcripts, encoding a shortened PK monomer due to a premature end of translation at residue 95. At the 5' splice site, the two main effects of the novel IVS4-2A>C base change were retention of IVS4 and the simultaneous skipping of both exons 5 and 6. Retention of IVS4 predicts the in-frame insertion of 32 additional amino acids between residues 125 and 126. Skipping of both exons 5 and 6 renders a transcript with a premature stop codon in exon 7. The main effect of the IVS11-3C>G mutation was a strongly reduced amount of transcripts. The remaining transcripts were processed normally or at an alternative donor site 5 nt upstream in exon 12. The novel c.990C>T base substitution in exon 8 does not change the codon for serine at residue 320. Interestingly, however, this mutation was associated with an increased amount of transcripts processed at an alternative donor site at nt 985. Consequently, this in-frame deletion would remove residues 329 to 372 from the PK monomer. Conclusions. The results of our studies provide insight into the molecular mechanisms by which the herein described mutations lead to PK deficiency. It shows in particular that any type of mutation may affect pre-mRNA processing. This will contribute to the better understanding of the pathophysiology of PK deficiency and, in general, the complex regulation of pre-mRNA processing.

ASSESSMENT OF COGNITIVE EFFECTS OF ONCE-WEEKLY EPOETIN ALFA IN ANEMIC PATIENTS WITH HEMATOLOGIC MALIGNANCIES RECEIVING CHEMOTHERAPY: RESULTS OF THE EPOLYM TRIAL

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Background. Increasing evidence suggests that chemotherapy can produce cognitive dysfunction in cancer patients, while the cognitive deficits tend to be subtle, they can have a negative impact on the patients’ social, educational, and professional activities, and overall quality of life (QoL). Clinical evidence suggests an association between chemotherapy-related decreases in hemoglobin (Hb) level and an increased risk of cognitive dysfunction. Aims. To assess changes in cognitive function in patients undergoing chemotherapy and receiving once-weekly (QW) epoetin alpha to maintain Hb levels and prevent subsequent fatigue, symptoms of anemia and deficits in QoL. Methods. EPOLYM was a 24 week, prospective, international, multicenter, open-label, Phase IIIb trial in anemic (Hb <12.0 g/dl) patients receiving chemotherapy (N=1034) for Hodgkin’s disease (HD), non-Hodgkin’s lymphoma (NHL), chronic lymphocytic leukemia (CLL), and multiple myeloma (MM). Epo-
etin alfa therapy was initiated at a dose of 40,000 IU QW administered subcutaneously, with dosage adjustments to be made based on clinical response (target Hb, 11.5-13.0 g/dL). Cognitive function was evaluated at baseline, Weeks 1, 6, 12 and at 24 weeks or study completion. Summary statistics were calculated for each measure at each assessment. The Cognitive Drug Research (CDR) Computerised Cognitive Assessment System, a computerised battery of tests (tasks) performed by subjects, was used to assess changes in parameters of cognitive function, including tasks of attention (Simple Reaction Time, Choice Reaction Time, Digit Vigilance), working memory (Numeric Working Memory), and secondary memory (Immediate and Delayed Word Recognition, Picture Recognition). Because of the relationship between affect and cognitive, depression and anxiety were assessed at the time of cognitive assessment using the Hospital Anxiety and Depression Scale (HADS). Changes from baseline in Hb levels, transfusion requirements, and QOL measures were also evaluated (transfusion and QOL results not reported here). Results. Analyses were performed on the cognitive data (904/1034 patients) and HADS scores (978/1034 patients). Performance on attention tasks was slightly impaired over the duration of the study, reaching significant decrease from baseline at weeks 12 and 24 (p<0.05). Continuity of attention, the ability to sustain attention and avoid error, had a pattern of improvement from baseline over time with a significant improvement at week 12 (p=0.027). Speed of memory improved from baseline, achieving significance (p=0.005) at each evaluation point. HADS scores were near the high normal range at baseline and improved slightly from baseline during the study, reaching significance (p=0.001) from week 6 onward. The baseline up to week 24 significant improvement in HADS scores was associated with increase in Hb level, with those developing an Hb increase of >1 g/dL were the most improved HADS score. Similarly, the indications of clinical improvement in cognitive function were related to a significant (p<0.0001) increase in Hb from 10.4±1.3 g/dL at baseline to 12.0±1.7 g/dL at 24 weeks. Conclusion. Overall, the assessment of data indicated a positive change in cognitive function parameters and HADS scores over the 24 week study. These improvements were associated with an increase in Hb level achieved with QW epoetin alfa.

0022

DEFERASIROX (EXJADE, ICL670), THE NOVEL, ONCE-DAILY ORAL IRON CHELATOR, IS WELL TOLERATED AND EFFECTIVE IN TREATING TRANSFUSIONAL IRON OVERLOAD IN PATIENTS WITH A RANGE OF RARE ANAEMIAS

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Background. Deferasirox (Exjade®, ICL670), the novel, once-daily oral iron chelator, is currently approved for use in eight countries for the treatment of transfusional iron overload in patients aged ≥22 years. Deferasirox has been shown to be effective and well tolerated in patients with various transfusion-dependent anaemias, including β-thalassaemia. There are, however, a number of rare anaemias that may also require transfusion therapy, meaning that patients are at risk for iron overload. To date, little has been published regarding iron overload and chelation therapy in these rare anaemias. Aims. To evaluate the severity of iron overload, as well as the efficacy and safety/tolerability of deferasirox in transfusion-dependent patients with a range of rare anaemias (a subpopulation of a Phase II study). Methods. The overall study was an open-label, multicentre, 1-year trial that enrolled 22 patients with a range of rare anaemias, including: aplastic anaemia (n=5), α-thalassaemia (n=3), sideroblastic anaemia (n=3), myelofibrosis (n=2), pure red cell aplasia (n=2), pyruvate kinase deficiency (n=2), autoimmune haemolytic anaemia (n=1), Fanconi’s anaemia (n=1), hereditary sideroblastic anaemia (n=1), erythropenia (n=1), and unspeciﬁed anaemia (n=1). Patients were assigned deferasirox doses according to baseline liver iron concentration (LIC). Results. Enrolled patients (median age 52 years; range 4-77) received deferasirox 10, 20 or 30 mg/kg/day (n=1, 10 and 11, respectively). The median duration of exposure to deferasirox was 52.1 weeks (range 15.7-169). The median number of transfusions during study was 13.5 (25-75% percentiles: 6.0-18.0), while the median blood transfused was 0.31 ml red blood cells/kg/day (25-75% percentiles; 0.12-0.43). Mean baseline LIC in this sub-population was high (15.1 mg Fe/g dw; SD±6.2), but decreased by 3.7 mg Fe/g dw (SD±6.5) after 1 year of deferasirox treatment. Mean serum ferritin level at baseline was 3144 µg/L (SD±1850), and fell by 750 µg/L (SD±1517) during study. The mean rate of iron excretion (0.41±0.19 mg/kg/day) exceeded iron intake (0.31±0.19 mg/kg/day). Seventeen patients (77.3%) completed the study; three subjects discontinued as they no longer required study drug and two withdrew due to adverse events (AEs). There were no deaths in this patient subgroup. All 22 patients reported at least one AE, the majority of which were transient and mild to moderate in severity. The most common drug-related AEs were mild, transient gastrointestinal disturbances such as diarrhoea (n=5, 54.4%), nausea, vomiting (n=4, 18.2% for each) and abdominal pain (n=2, 9.2%). Mild, non-progressive serum creatinine increases ≥33% of baseline were observed in 12 patients receiving deferasirox 20 and 30 mg/kg/day (within the normal range in seven patients, >ULN in five). There were no incidences of drug-induced neutropenia or arthralgia. Conclusions. In these patients with diverse rare anaemias, deferasirox was well tolerated and was above the published clinically acceptable thresholds. This suggests that these patients are at increased risk for developing co-morbidities with a resultant negative impact on survival. Once-daily, oral deferasirox was effective and generally well tolerated, resulting in a clinically relevant reduction in overall body iron burden.

0023

GLYCOCALYX PERTURBATION IN PATIENTS WITH SICKLE CELL DISEASE: IMPLICATIONS FOR VASCULAR VULNERABILITY

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Background. Activated endothelium plays a pivotal role in the pathogenesis of sickle cell disease (SCD). The activation of the endothelium is caused by hypoxia, reperfusion damage, high shear rates and pro-inflammatory mediators, like thrombin and TNF-α. The central role of the glyocalyx (a layer of hyaluronan and proteoglycans covering the endothelium) has been established as an antiadhesive and antithrombotic endothelial barrier. Recently, we have validated a technique to determine the endothelial glyocalyx in human subjects and demonstrated that the glyocalyx volume is strongly diminished in diabetic patients with microangiopathy. Aim. Sickle cell patient are known to have a strongly activated endothelium and may also develop microangiopathy. Therefore, we assessed the glyocalyx volume in patients with SCD in comparison with carriers of SCD. Methods. The gly-
cocalyx was measured in 20 patients with SCD (HbSS, HbSC and HbSB) and 10 sex- and age-matched carriers of SCD. We determined the total systemic glycocalyx volume by comparing the intravascular distribution volume of a glycocalyx permeable tracer (dextran 40) to that of a glycocalyx impermeable tracer (autologous labelled erythrocytes).

**Results.** Patients with SCD demonstrated to have a significant reduced glycocalyx volume of 0.48±0.14 litres as compared to 1.26±0.27 litres in the carriers of sickle cell disease (p=0.009, expressed as mean±SEM). However, no correlation between glycocalyx volume and microangiopathy, disease severity or genotype was found. **Conclusions.** The strongly diminished glycocalyx volume in sickle cell patients resembles the chronic state of activation of the endothelium in SCD that may also be responsible for the enhanced adhesion of leukocytes and erythrocytes as well as the prothrombotic state of these patients. Since the glycocalyx layer serves as an important barrier between the endotherium and the circulating blood cells to prevent the adhesion of leukocytes, therapies that may restore or preserve glycocalyx function are warranted in SCD.

Figure 1. Glyocalyx volumes in litre (mean and SEM).

### 0024
**THE DIFFERENTIAL DIAGNOSIS OF INHERITED SIDEROBLASTIC ANAEMIA**

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**Background.** Over two decades peripheral blood or genomic DNA samples have been referred to University Hospital of Wales for molecular diagnosis of inherited or congenital sideroblastic anaemia (SA). **Aims.** The aim was to review the haematological and additional features of referred cases, to discover the proportion of cases diagnosed, and to critically analyse the features of those cases that remain undiagnosed, in order to identify developmental needs. **Methods.** Routine measurements carried out in our laboratory include FBC, reticulocyte counts, total erythrocyte free (FPP) and zinc protoporphyrin (ZPP), haemoglobinopathy screen, measurement of iron stores and gene sequence analysis of ALAS2, ABC7 and on some occasions FECH. In addition HFE genotyping, erythrocyte mRNA, X chromosome inactivation ratios, globin gene analysis and tissue culture techniques are employed. **Results.** Altogether there are 71 probands (51M, 20F), aged 1yr to >80yr, and 78 relatives of 58 families from 24 different sources around the world. Three were later reported as having other causes including one with Pearson's syndrome. Two were found to have undiagnosed homozygous α thalassemia (HbQuong Sze) and were not further investigated. One was not known to have SA and was α3.7kb/α3.7kb with a lower than expected MCHC. Two others with SA were previously thought to have thalassemia and one was shown to be α3.7kb/α3.7kb. In those with raised erythrocyte protoporphyrin levels (22/50) for whom composition was determined (x10) most showed mainly raised free PP (x13) and only four showed significantly raised zinc PP with absent or only slightly increased free PP. Two had FPP levels in the region expected for erythropoietic protoporphyria (EPP). ALAS2 mutations shown not to be common polymorphisms were found in 25: one new putative CATA1 site 5 nt deletion in the promoter region, two new putative splice site mutations, one single amino acid deletion, and 22 with 16 different missense mutations of which 4 have not yet been described (ile324Thr; Arg368Try; Ser521Phe). Three anaemic female probands carrying novel ALAS2 variations were shown to have skewed X chromosome inactivation of their buffy coat DNA (two have probable severe variations with macrocytic red cells and one with microcytic red cells responded initially to pyridoxine but seems now to be pyridoxine-refractory) and three haematological carriers showed balanced X chromosome inactivation. Variations were found in only four undergoing ABC7 investigations: the published Val411Le in two brothers (two with raised ZPP) and a maternal uncle with cerebellar ataxia, and a consensus GT→AT splice-site variation of unknown importance in a female heterozygote with severe anaemia. In those patients with markedly raised free erythrocyte PP for whom FECH was examined, only one with values in the EPP region was found to be a double heterozygote for a missense mutation of uncertain importance and the common low-expression allele. **Summary/Conclusions.** These studies extend the range of SA-associated variations, confirm the heterogeneity of presentation and causes and demonstrate a reasonable success for the current diagnostic strategy (approximately 50%). Those who remain undiagnosed remain heterogeneous in presentation requiring new tools to probe the emerging candidate pathways.

### 0025
**INTRAVENOUS ZOLEDRONIC ACID TREATMENT IN THALASSAEMIA-INDUCED OSTEOPOROSIS: RESULTS OF A PHASE II CLINICAL TRIAL**

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**Background.** Osteoporosis is an important cause of morbidity in β-thalassemia patients. Bisphosphonates have been recently used for the treatment of osteoporosis in β-thalassemia. **Aims.** This study is a prospective quasi-experimental study to assess the efficacy and safety of zoledronic acid in thalassemia patients with osteoporosis. The aim of the study is to assess the efficacy and safety of zoledronic acid, administered at a dose of 4 mg intravenously every 3 months over a period of 12 months to patients with β-thalassemia and osteoporosis. **Methods.** Eighteen thalassemia patients with osteoporosis were given zoledronic acid 4 mg intravenously every 3 months over a period of 12 months. The efficacy of treatment was assessed by measuring Bone Mineral Density (BMD) at the lumbar spine, femoral neck and hip at baseline, 6 and 12 months. Z-score was used to measure the BMD. Other medical assessments included markers of bone formation and resorption (bone alkaline phosphatase (BAP), osteocalcin (OC), and urinary deoxypyridinoline (Dpd)), and the assessment of pain score, analgesic score, and performance score. Ten thalassemic osteoporotic patients were followed up only with serial BMDs as controls.

**Table 1. BMD values of treatment and control group.**

<table>
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<th>Treatment Means±SD (Z-score)</th>
<th>p-value</th>
<th>Control Means±SD (Z-score)</th>
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<tr>
<td>baseline</td>
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<td>6 months</td>
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<tr>
<td>12 months</td>
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<td>-2.92±0.76</td>
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<tr>
<td>Femoral Neck</td>
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<td></td>
</tr>
<tr>
<td>12 months</td>
<td>-1.34±0.68</td>
<td></td>
<td>-1.70±0.35</td>
<td></td>
</tr>
</tbody>
</table>

**Results.** Both groups had no significant difference with respect to age, gender and baseline BMD. Patients taking zoledronic acid had a significant increase in their lumbar spine, femoral neck, and total hip BMD.
measurements over the 12-month period. Patients in the control group did not have any significant change in BMD measurements. Table 1 shows the BMD values of the treatment and control groups. There was a significant change in the levels of OC and BAP over the 12-month follow-up. There was a significant decrease in the number of painful sites experienced by the patients over the whole treatment period (p<0.01). Pain and analgesic scores significantly decreased over the whole treatment period (p=0.00 and 0.01 respectively). Pain interference with general activity and ECOG score, on the other hand, did not show any significant change. Reported adverse events included joint pain in 9 patients (50%) after the 1st dose and in 2 (11.1%) after the 2nd dose and responding very well to oral analgesics. Two patients (11.1%) had peripheral numbness and 3 (16.7%) had low grade fever after the 1st dose. No treatment-related adverse events were reported after the 3rd and 4th doses. None of the patients experienced elevated serum creatinine levels and none discontinued the study. Conclusions. Treatment of thalassemic osteoporotic patients with zoledronic acid, administered at a dose of 4 mg intravenously every 4 months over a period of 12 months, is safe and very effective in increasing BMD at the lumbar spine and hip and in reducing pain and is well-tolerated.

0026
THE DISTINCTION BETWEEN HAEMOLYSIS DUE TO HEREDITARY SPHEROCYTOSIS AND THAT DUE TO A CATION PERMEABILITY DISORDER OF THE RED CELL MEMBRANE IN A REGULAR HAEMATOLOGY LABORATORY
J.S. Goede,1 R. Reggi,1 G.W. Stewart,2 P. Harrison,3 H.C. Robinson,4 T. Latshang,5 J. Fehr,5 J.C. Ellory,5 H.U. Lutz5
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Background. An increased fraction of hyperchromic RBCs, reticulocytosis, splenomegaly and reduced osmotic resistance of RBCs leads in 1.5), macrocytic erythr (80-100), MCHC 34.7g/dL (31-36), hyperchromic erythrocytes 0.1% (0-
values: Haemoglobin 15.6g/dL (normal range 13.5-17.2), MCV 105.5fl (80-100), MCHC 39.2 g/dL (31-36), hyper-
1.5). Analysis after storage on ice for two hours showed the following

0027
RITUXIMAB AND FLUDARABINE COMBINATION THERAPY FOR CHRONIC COLD AGGLUTININ DISEASE
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Background. Primary chronic cold agglutinin disease (CAD) is an autoimmune haemolytic anaemia characterized by the production of monoclonal antibodies, most often IgM kappa, against erythrocyte surface antigens. A clonal lymphoproliferative bone marrow disorder can be demonstrated in most cases. Rituximab single agent therapy has been shown in prospective studies to induce remission in more than 50% of patients. Aim. We wanted to improve on response rates achieved by therapy directed against the underlying clonal B cell proliferation. Methods. In a prospective phase II trial, eligible CAD patients received rituximab 375 mg/sqm intravenously d 1, 29, 57 and 85, and fludarabine tablets 40 mg/sqm d 1-5, 29-33, 57-61 and 85-89. Clinical, haematological, immunological and histological data were recorded, and responses were classified according to previously published criteria as complete (CR), partial (PR), or no (NR) response. Results. By Feb 2006 we have treated six patients with a median age of 70. All patients had monoclonal IgM kappa and considerable or severe cold-induced circulatory symptoms. All had been previously treated with rituximab single agent therapy, resulting in one CR, one PR and four NR. After the combination therapy, circulatory symptoms resolved completely in four patients and improved in one additional patient. Haemoglobin levels increased by > 3 g/dL in two of four anaemic patients. Overall, two patients achieved CR, two achieved PR while two were non-responders. Haematological toxicity was recorded in three patients (grade 2, 3 and 4, respectively), infection grade 2 in one and nausea in one. Conclusions. Rituximab and fludarabine combination therapy is feasible even in elderly patients with CAD. Response rates are promising, but superiority over rituximab single agent therapy remains to be proven until more patients have been treated.

Table 1.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Indication for therapy*</th>
<th>Bone marrow histology**</th>
<th>Hb g/dL</th>
<th>Increase in Hb g/dL</th>
<th>Change in circulating symptoms</th>
<th>Overall response</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>59</td>
<td>F</td>
<td>HA,CS</td>
<td>LPL</td>
<td>9.9</td>
<td>3.8</td>
<td>Resolution</td>
<td>CR</td>
</tr>
<tr>
<td>2</td>
<td>77</td>
<td>F</td>
<td>CS, HA</td>
<td>UCL</td>
<td>10.3</td>
<td>0.3</td>
<td>Improvement</td>
<td>NR</td>
</tr>
<tr>
<td>3</td>
<td>66</td>
<td>M</td>
<td>CS</td>
<td>LPL</td>
<td>12.2</td>
<td>1.0</td>
<td>Resolution</td>
<td>PR</td>
</tr>
<tr>
<td>4</td>
<td>74</td>
<td>M</td>
<td>CS</td>
<td>LPL</td>
<td>16.0</td>
<td>0.3</td>
<td>Resolution</td>
<td>CR</td>
</tr>
<tr>
<td>5</td>
<td>62</td>
<td>F</td>
<td>CS, HA</td>
<td>LPL</td>
<td>10.4</td>
<td>3.1</td>
<td>Resolution</td>
<td>PR</td>
</tr>
<tr>
<td>6</td>
<td>85</td>
<td>M</td>
<td>HA,CS</td>
<td>LPL</td>
<td>7.8</td>
<td>1.16</td>
<td>No change</td>
<td>NR</td>
</tr>
</tbody>
</table>

*HA, haemolytic anaemia; CS, circulatory symptoms; **LPL, lymphoplasmacytic lymphoma; UCL, unclassified clonal lymphocytosis.

0028
IN VIVO OXIDATIVE ERYTHROCYTE MEMBRANE PROTEIN DAMAGE IN HEREDITARY SPEROCYTOSIS
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Background. Hereditary spherocytosis (HS) is a heterogeneous group of disorders with regard to clinical severity, gene defects and mode of inheritance. Most patients are presented with mild or moderate hemolysis. The abnormal red cell morphology (resulting in shortened cell survival) is due to a deficiency of, or a dysfunction in, spectrin, ankyrin, band 3 or palladin. Previous in vitro studies suggested that the spherocytes are sensitive to the action of oxidative agents. Furthermore, higher Hb autooxidation rate and abnormal oxidant sensitivity of spectrin, have also been reported in HS. Aims. To determine the possible oxidation-related protein alterations and the oxidative index of the membrane ghosts and cytoskeletons in clinically diagnosed cases of HS. Methods. Twelve patients with clinical and laboratory diagnosis of mild to moderate HS [ank(-)HS N=4, Sp(-)HS N=3, B3(-)HS N=5, splenectomized N=2, concomitant carriers of α- or β-thalassemia N=4] and twelve healthy subjects used as controls were examined. Total ghosts and cytoskeletons were analyzed by SDS-PAGE densitometry and probed for

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hemoglobin, human immunoglobulins (IgG’s) and various membrane proteins using erythroid specific antibodies. Carbonylated protein content was determined following 2,4-dinitrophenylhydrazine derivatization and SDS-PAGE coupled with western blotting with anti-DNP moiety antibody. Results. Protein degradation, formation of high molecular weight aggregates and increased Hb and IgG’s binding to the membrane were found by means of SDS-PAGE and immunoblotting analysis in the majority of the HS patients examined. The protein band-8 (22 kDa) was increased in 8/12 patients, half of which had concomitantly increase in Hb. Probing of the HS ghost membranes for Hb clarified that the membrane-associated globin was in the form of probably oxidized/denatured Hb or hemichromes. Subsequent analysis of the Triton-extracted membrane skeletons revealed pathologically increased amounts of skeleton-associated Hb monomers and higher order aggregates, representing globin oligomers and complexes with membrane protein components, in 30% of the samples. Immunoblotting with dinitrophenol-specific antibody showed increased RBC membrane and cytoskeleton protein carbonyls in the majority of the HS patients. In comparison to control membranes, there was an evident increase in the number and the intensity of the carbonylated protein bands appearing in the immunostained gels, ranging from MW 240 kDa to 15 kDa, in approximately 70% of the HS samples that were examined.

Summary/Conclusions. The red cells in HS in vivo are characterized by oxidative alterations in Hb and various membrane proteins and increased degradation in levels. Similar defects in this pool of membrane and intracellular membranes are also found in vivo stored and senescent RBCs are dictated by increased oxidative stress and are positively correlated with perturbations in membrane properties. These data corroborate the evidence for the occurrence of oxidative damage in membrane proteins in HS and add some new insight in the field of HS pathophysiology.

**Table 1. Study endpoints.**

<table>
<thead>
<tr>
<th></th>
<th>IV iron</th>
<th>Standard practice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BL Hb &lt;10 g/dL</td>
<td>BL Hb ≥10 g/dL</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>9.3 (5.4)</td>
<td>10.6 (3.9)</td>
</tr>
<tr>
<td>Crude % (95% CI)</td>
<td>19 (5 to 42)</td>
<td>29 (13 to 51)</td>
</tr>
</tbody>
</table>

Note: 115 pts were randomised, but 1 pt in the IV iron group was not treated.

*Based on the number of pts (for IV iron, n=21 for BL Hb <10 g/dL and n=34 for BL Hb ≥10 g/dL; for standard practice, n=24 for BL Hb<10 g/dL and n=30 for BL Hb≥10 g/dL) who were in the study until at least Day 29.

**B3030**

**CHARACTERISATION OF INDIVIDUAL NADH-CYTOCHROME B5 REDUCTASE VARIANTS USING A HETEROLOGOUS EXPRESSION SYSTEM**

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Background. Recessive congenital methaemoglobinemia (RCM) arises from deficiency of NADH-cytochrome b5 reductase (cb5r) and manifests as cyanosis from birth. It exhibits two clinical phenotypes, benign type I and more severe type II, where the cyanosis is associated with neurological impairment. The physiological basis for the phenotypic variation between type I and type II RCM is poorly understood. Several mutations, Arg159del and Val252Met, have been found associated with both types suggesting that it is the combination of both alleles and thus the residual activity of cb5r variants that influences the development of type II as opposed to type I RCM. To date more than 40 mutations of cb5r have been described with a cluster in exon 9 of the DIA1 gene. To characterise individual cb5r variants a heterologous expression system has been developed based upon the X-ray crystallography structure of the rat cb5r protein. Expressed proteins can then be purified to homogeneity and investigated for protein stability, catalytic efficiency, EAD cofactor properties and NADH/ferricyanide and NADH:cytochrome b5 reductase activities, thermostability measurements and dye-mediated redox titrations of the EAD prosthetic group. Results. Four of the expressed variants, Gly75Ser, Asp239Gly, Val252Met, Pro275Leu and Gly291Asp, recently described by 79% of patients in the IV-iron group and 2% of patients in the standard-practice group had embolic/thrombotic events. Haemoglobin and transfusion endpoints stratified by baseline-haemoglobin category are shown in the table. Summary/Conclusions. Based on the interim results, the safety profile for patients receiving 500-mcg darbepoetin alfa Q3W with IV iron appears to be comparable to patients receiving 500-mcg darbepoetin alfa Q3W with oral iron or no iron. The percentage of patients who achieved the target haemoglobin (≥11 g/dL) appeared higher, and the percentage of patients who required transfusions appeared lower, in the group receiving IV iron. This trend was consistent in patients in both baseline-haemoglobin groups.
exhibit decreased enzyme activity when compared to the recombinant wild type cb5r protein. The Arg159del variant protein was unstable and could not be purified thus preventing further characterisation. Although four variants, Gly75Ser, Val252Met, Pro275Leu and Gly291Asp, exhibited impaired protein stability the Asp239Gly had near wild type protein stability. A reduction of 40-fold and 487-fold respectively in the affinity of cb5r towards NADH co-factor was found in the Gly75Ser and Pro275Leu variants. Using predictions from the rat model, residue Asp239 is essential for the selection of NADH over NADH and Pro275Leu is the main residue required for positioning cb5r to allow binding to the NADH substrate. Although Gly75 is present in a highly conserved area of the FAD-binding lobe of cb5r it appears to influence NADH affinity as the Gly75Ser variant exhibited an increase in affinity for NAD+. Summary. The heterologous expression system has been a useful tool for providing insights into the impact of type I RCM mutations on the structure and function of cb5r. It may allow the relationship between the clinical phenotype and cb5r activity to be examined and may lead to better understanding of the pathophysiology of the two types of RCM.

0031
DEFERASIROX (EXJADE, ICL670) PROVIDES 24-HOUR PROTECTION FROM LABILE PLASMA IRON (LPI), IN IRON OVERLOADED β-TALASSAEMIA PATIENTS PREVIOUSLY CHELATED WITH MONO- OR COMBINATION THERAPY
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‘Sultan Qaboos University, MUSCAT, Oman; 1American Univ Beirut-Chronic Care Center, BEIRUT, Lebanon; 2Novartis Pharma AG, BASEL, Switzerland
Background. Chelation therapy aims to reduce iron burden, as patients are at increased risk for developing co-morbidities with a resultant negative impact on survival unless excess iron is removed. This can be achieved by reducing free or non-transferrin bound iron (NTBI). LPI, one form of NTBI, is redox-active and can be taken up by cells, resulting in expansion of the cellular iron pool and an increased propensity for radical formation with ensuing oxidative stress. Direct capture of LPI has been suggested to avoid accumulation of cellular iron and to prevent its adverse consequences. Aims. To evaluate baseline data from the ongoing ESCALATOR trial and to measure LPI change in a patient subgroup. The overall aims of the ESCALATOR study are to investigate the efficacy and safety of the novel, once-daily iron chelator deferasirox (Exjade®, ICL670; 20 mg/kg/day) in 232 iron overloaded β-thalassaemia patients previously chelated with mono-or combination therapy. Methods. Patient characteristics (159 aged 2-15 years; 73 aged ≥16 years) were analyzed to determine baseline iron burden. Pre-administration and 2-hour post-administration LPI levels were measured in a subgroup of 14 patients, at baseline and following repeat administration (weeks 4 and 16). Results. Despite previous chelation, baseline iron burden in the overall population was high, indicating severe iron overload; mean baseline liver iron concentration (LIC) was 18.0 mg Fe/g dw (SD±9.1) and serum ferritin was 4146 µg/L (SD±2319), with both measures greater in adult than paediatric patients. Baseline LIC and serum ferritin values were well correlated (R=0.63), supporting serum ferritin as a surrogate marker of body iron burden. In the LPI subgroup, all of whom had received combination therapy with deferoxamine and deferoxamine, baseline iron burden was also high (LIC 30.0 mg Fe/g dw; range 11.5-48.9).

Table 1. LPI, pre and post deferasirox administration, at baseline and after repeat administration.*

<table>
<thead>
<tr>
<th>LPI, mmol/L</th>
<th>Baseline (n=13)</th>
<th>Week 4 (n=13)</th>
<th>Week 16 (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>Mean ± 50</td>
<td>0.99±0.82</td>
<td>0.12±0.16</td>
<td>0.08±0.29</td>
</tr>
<tr>
<td>Pre vs post</td>
<td>p&lt;0.0001</td>
<td>p&lt;0.0119</td>
<td>p&lt;0.1948</td>
</tr>
<tr>
<td>Baseline</td>
<td>p=0.0187</td>
<td>p=0.0007</td>
<td></td>
</tr>
</tbody>
</table>

Although baseline LPI levels were high, Table 1 demonstrates a significant reduction in post- versus pre-administration levels at baseline and week 4. Pre-administration LPI levels were within normal parameters (0-0.4 µmol/L) by week 4, and were further reduced by week 16; post-ver-

RETAINED FOR REVIEW
**Drug resistance & drug pharmacology**

### 0033 GENETIC AND LEUKAEMIA-SPECIFIC FACTORS ASSOCIATED WITH P-GLYCOPROTEIN EXPRESSION AND FUNCTION IN AML BLASTS

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1Nottingham City Hospital, NOTTINGHAM, United Kingdom; 2Cardiff University, CARDIFF, United Kingdom

Background. P-glycoprotein (pgp), expressed on acute myeloid leukaemia (AML) blasts, is associated with failure to respond to chemotherapy in AML. Aims. This study aimed to determine whether expression and function of pgp may be linked to polymorphisms of the encoding gene (MDR1, also known as ABCB1) and whether leukaemia-specific changes in cell biology may override genetic factors in predicting pgp expression. Methods. G1199A, G2677T and C3435T polymorphisms in MDR1 (RFLP analysis) as well as pgp protein expression (using MRK-16 and function (modulation of R125S accumulation by PSC 833) were studied in leukaemic blast samples from 631 patients with AML entered into the NCRM AML14 and AML15 clinical trials. Results. 41.7% cases had functional pgp; 42.9% had intermediate/high protein expression; 9.5% had function with undetectable/low protein expression; 12.7% had intermediate or high protein expression with no function and 48% had both low/negative protein and function. At position G1199A there were 5.7% heterozygotes and 0.5% A variant homozygotes. At position G2677T there were 45.7% heterozygotes and 21% T variant homozygotes. At position C3435T there were 46.7% heterozygotes and 30.5% TT variant homozygotes. In a subset of 316 patients, on whom complete data were available, further analysis was performed. The C3435T and G2677T gene polymorphisms affected pgp protein expression, with the lowest protein expression occurring in the variant TT group for both polymorphisms (using Kruskal-Wallis test, p = 0.005 and 0.006 respectively), but there was no significant association between polymorphisms and pgp function. The I199 polymorphism did not affect protein expression or function. Linkage disequilibrium occurs between positions 2677 and 3435. There was a highly significant association between the haplotype and pgp protein expression (p = 0.005). The variant (v/v) homozygote haplotype expressed the least pgp protein (p = 0.001). MDR1 mRNA was also measured in 81 patients. Message, protein and function were highly correlated (p < 0.001 for each comparison). Biological factors were also analysed. The phenotypic and genotypic factors associated with pgp protein expression are shown in the table. In univariate analysis, white blood cell count, cytogenetic risk group, age at diagnosis, secondary AML/MDS and MDR1 haplotype were all strongly associated with pgp protein expression. In multivariate analysis white blood cell count, cytogenetic risk group, age and MDR1 haplotype retained significance. Cell cycle analysis of 39 consecutive high protein expression. In multivariate analysis white blood cell count, cytogenetic risk group, age and MDR1 haplotype retained significance. Cell cycle analysis of 39 consecutive high protein expression. In multivariate analysis white blood cell count, cytogenetic risk group, age and MDR1 haplotype were all strongly associated with pgp protein expression. In multivariate analysis white blood cell count, cytogenetic risk group, age and MDR1 haplotype were all strongly associated with pgp protein expression. In multivariate analysis white blood cell count, cytogenetic risk group, age and MDR1 haplotype we

Table 1. Factors associated with pgp protein expression.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Neg/low pgp protein (n=32)</th>
<th>High pgp protein (n=57)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median WBC (&lt;10 × 10⁹/L)</td>
<td>12.5</td>
<td>12.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Median age (years)</td>
<td>57</td>
<td>63</td>
<td>0.008</td>
</tr>
<tr>
<td>Good risk cytogenetics</td>
<td>68%</td>
<td>32%</td>
<td>0.001</td>
</tr>
<tr>
<td>Intermediate risk cytogenetics</td>
<td>63%</td>
<td>37%</td>
<td>0.008</td>
</tr>
<tr>
<td>Poor risk cytogenetics</td>
<td>36%</td>
<td>65%</td>
<td>0.000</td>
</tr>
<tr>
<td>De novo AML</td>
<td>62%</td>
<td>39%</td>
<td>0.008</td>
</tr>
<tr>
<td>Secondary AML</td>
<td>36%</td>
<td>64%</td>
<td>0.001</td>
</tr>
<tr>
<td>MDS</td>
<td>25%</td>
<td>75%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>26/77/3435 haplotype 23 WT allele</td>
<td>56%</td>
<td>44%</td>
<td>0.000</td>
</tr>
<tr>
<td>26/77/3435 haplotype V4/V4</td>
<td>78%</td>
<td>22%</td>
<td>0.002</td>
</tr>
</tbody>
</table>

### 0034 FCGRIIIA 158 V/V GENOTYPE IS ASSOCIATED WITH INFERIOR RESPONSE TO RITUXIMAB AND CHOP IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA

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Background. Patients with follicular lymphoma or Waldenstrom’s macroglobulinaemia and a homozogous valine/V(valine (V) at position 158 of the FcγRIIIa (CD16) receptor have a superior response to rituximab monotherapy. This could be related to higher affinity binding of IgG1 and NK cells with FcγRIIIA 158 V/V genotype to an IgG1, suggesting antibody dependent cell-mediated cytotoxicity (ADCC) as an important mechanism of rituximab action in indolent lymphoma. Similarly, a histidine(H)/arginine(R) dimorphism in position 131 of the FcγRIIa (CD32) may also be related to the treatment response. There is no data whether these dimorphisms affect response to combination of rituximab and chemotherapy in aggressive lymphoma. Aims. We examined the correlation of FcγRIIIa and FcγRIIa gene dimorphisms with response in patients with diffuse large B-cell lymphoma (DLBCL) treated with rituximab and CHOP (R-CHOP). Methods. FcγRIIIa and FcγRIIa gene dimorphisms were determined in 46 previously untreated patients with DLBCL presenting with stage IV in the R-CHOP regimen. Genotyping of FcγRIIIa 131 histidine/arginine (R) and FcγRIIa 158 valine/V/phenylalanine (F) was performed by PCR followed by allele-specific restriction enzyme digestion. Response to R-CHOP was analyzed according to standard criteria. Complete or unconfirmed complete remission (CR) after R-CHOP was achieved in 74% patients (34/46). Three patients achieved partial remission and nine patients did have no response to the treatment. The frequency of FcγRIIIa 158 V/V, V/F and F/F was 22%, 63%, 15%, respectively. The frequency of FcγRIIa 131 H/H, H/R, R/R was 39%, 48%, 18%. There was no difference in age, sex or IPI between the groups. Surprisingly, patients with FcγRIIIa 158 V/V genotype had significantly lower CR rate compared to FcγRIIIa 158 F carriers (40% vs. 82%, p = 0.011). There were no significant differences in CR rate between the patients with different FcγRIIIa 131 genotypes. Summary/conclusions. Contrary to the previous reports of response to rituximab in follicular lymphoma, FcγRIIIa 158 V/V genotype in DLBCL was associated with significantly lower response rate to R-CHOP therapy. These results support the hypothesis that antibody dependent cytotoxicity (ADCC) does not mediate rituximab activity in DLBCL. Some other mechanisms, such as chemosensitization or direct apoptosis may be involved in synergistic effect of rituximab and CHOP in DLBCL. Considering the wide inter-individual variability in pharmacokinetics of rituximab, it is possible that FcγRIIIa 158 V/V genotype provides more effective elimination of rituximab overcoming the hypothetical benefit of ADCC in patients with DLBCL treated with R-CHOP.

### 0035 DEFINED BONE MARROW NICHE COMPONENTS MEDIATE THE IN VITRO RESISTANCE OF AML SAMPLES TO THE TYROSINE KINASE INHIBITOR AG1296 AS WELL AS TO CYTOSINE ARABINOSIDE

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Background. Patients with AML tend to respond well to remission induction chemotherapy, but relapse is frequent, suggesting protection of minimal residual disease cells in the bone marrow niche. Aims. We sought to determine the effect of defined bone marrow niche components fibronectin and cytokines - on the survival and chemoresistance of AML cells. Methods. We examined the effects of the cell adhesion substrate fibronectin and/or the cytokines IL3, IL6, SDF-1, angiopoietin 1, stem cell factor (SCF) and several combinations to the in vitro adhesion, survival and response to the tyrosine kinase inhibitor AG1296 and to the nucleoside analogue cytosine arabinoside (ara-C) in 48 h suspension culture of presentation samples from AML patients. 12 of the 16 samples selected for study had internal tandem duplication of the FLT3 gene, since relapse rates are high in these patients and tyrosine kinase inhibitors (TKIs), is an attractive therapeutic strategy. Results. In vivo addition of fibronectin to primary AML samples at 2 hours was enhanced by co-culture with the cytokines IL-3 (69% increase, p = 0.04) and stem cell factor (89% increase, p = 0.04). In vitro survival at 48 hours in serum-free sus-
pension culture was enhanced 25% by adhesion to fibronectin (p=0.02, n=10) and further enhanced 32% by IL-3 (p=0.03), and 44% by a four cytokine cocktail (IL-3, IL-6, SCF and angiopoietin 1, p=0.02), but not by SCF, IL-6 or angiopoietin 1 individually. *in vitro* resistance to 15 μM AG1296 was enhanced 18% by adhesion to fibronectin (n=10, p=0.007) and further enhanced 45% by IL-3 (n=10, p=0.005), 57% by IL-6 (n=6, p=0.004) and 60% by the four cytokine cocktail (n=6, p=0.012). Similar- ly, *in vitro* chemoresistance to 500 ng/mL ara-C was enhanced 25% by adhesion to fibronectin (p=0.008, n=10) and further enhanced 31% by IL-3 (n=10, p=0.05) and 93% by the four cytokine cocktail (n=8, p=0.017). Conclusion. Adhesion to fibronectin increases survival and chemoresistance in AML samples and these effects are enhanced by cytokines, particularly IL-3. The tyrosine kinase inhibitor AG1296 and the cytotoxic drug cytostatin arabinoside evoked similar patterns of resistance. It may be necessary to target cell-adhesion-mediated mechanisms of drug resistance in order to prevent relapse in AML.

**0036**

**OVERCOMING CHEMORESISTANCE IN HUMAN CHRONIC MYELOID LEUKEMIA K562 CELLS BY SiRNA INHIBITION OF SHINGOSINE KINASE-1**

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Background. Sphingosine-1-phosphate (S1P), the product of sphingo- sinesis kinase-1 (SK-1), has been implicated as a second messenger that acts as a survival signal. Inhibition of SK-1 expression by siRNA, confirmed by decreased levels of mRNA results in the accumulation of ceramide and sphingosine which acts as pro-apoptotic signals and creates a survival signal. Inhibition of SK1 by RNAi results in the accumulation of ceramide and sphingosine which induce apoptosis. *Aims*. In this study, the imatinib resistant Ph(+) human CML cells were tried to be sensitized to imatinib by targeting the SK1 gene. *Methods*. The Ph(+) human K562 cells were exposed to step-wise increasing concentrations of imatinib. Subpopulations of cells that were able to grow in the presence of 0.2 and 1 μM imatinib, were then selected, and referred to as K562/IMA-0.2 and K562/IMA-1, respectively. Plasmid and siRNA transfection of K562 cells were conducted using an Effective and DharmaFECT(™) siRNA transfection reagent, respectively. Caspase-3 activity was determined using caspase-3 colorimetric assay. The mitochondrial membrane potential (MMP) was measured using a JC-1 MMP detection kit. The cellular levels of endogenous ceramides were measured using high performance liquid chromatography/mass spectrometry (LC/MS). The expression analysis of SK-1 was determined by RT-PCR and western blotting. *Results*. Measurement of the levels of SK-1 by RT-PCR and Western blotting, demonstrated that the expression of SK-1 is increased about 2- and 4-fold in K562/IMA-0.2 and K562/IMA-1 cells, respectively, when compared to controls. The possible role of SK1 in resistance to imatinib was further examined by the overexpression of SK1, which increased S1P levels, and prevented apoptosis significantly in sensitive K562 cells in response to 500 nM imatinib at 48 hr. On the other hand, in resistant K562/IMA-0.2 and K562/IMA-1 cells, partial inhibition of SK1 expression by siRNA, confirmed by decreased levels of endogenous S1P, increased sensitivity to imatinib-induced apoptosis. *Summary/Conclusions*. Targeting SK1 pathway, in addition to BCR/ABL kinase inhibition, increased the sensitivity of K562/IMA-0.2 and K562/IMA-1 cells to imatinib while the overexpression of SK1 increased the resistance in sensitive cells. It was shown that one of the mechanisms responsible for imatinib resistance may be increased amounts of S1P in resistant cells as compared to parental sensitive cells.

**0037**

**DEMONSTRATION OF SYNERGISTIC GROWTH-INHIBITORY EFFECTS OF DASATINIB AND CLADRIBINE IN NEOPLASTIC HUMAN MAST CELLS**

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In the majority of patients with systemic mastocytosis (SM) includ- ing aggressive SM and mast cell leukemia (MCL), neoplastic cells display the D816V-mutated variant of KIT. KIT-D816V exhibits constitutive tyrosine kinase (TK) activity and has been implicated in malignant cell growth. Whereas wild type (wt) KIT and several non 816-codon KIT-mutants, like KIT-G560V, are sensitive to the TK inhibitor imatinib, KIT- D816V confers resistance against this drug. Therefore, several attempts have been made to identify TK inhibitors able to counteract the novel TK inhibitor dasatinib (RMS-354825) counteracts TK activity of wt-KIT; KIT-G560V, and KIT-D816V as determined by western blotting. Dasatinib was also found to counteract viability of Ba/F3 cells expressing wt-KIT or KIT-D816V, as well as growth of HMCL-1 cells (KIT-D816V-negative) and HMC-1 cells (KIT-D816V-positive), whereas as imatinib did not counteract growth of KIT-D816V-positive cells over the dose range tested (0.1-10 μM). In all cells examined, the effects of dasatinib were dose-dependent, with 100-1,000-fold higher IC50-values found in cells harboring KIT-D816V compared to cells lacking KIT- D816V as assessed by SH-Tyminidine uptake. The growth-inhibitory effects of dasatinib were found to be associated with induction of apoptosis in HMC-1 cells (determined by conventional and electron microscopy) and by TUNEL-assay. Moreover, in this cell line, dasatinib was found to downregulate the expression of CD2 and CD68, two activation-linked cell surface antigens that are typically overexpressed on mast cells in SM as assessed by flow cytometry. One strategy to optimize the treatment of SM and to overcome drug-resistance might be to combine TK inhibitors with other (targeted or conventional) drugs. We there- fore investigated potential cooperative drug interactions between dasa- tinib and cladribine (2CdA), a cytochrome agent used for the treat- ment of SM. Dasatinib was found to synergize with 2CdA in counter- acting growth of neoplastic mast cell lines. In summary, our data show that dasatinib as a single agent or in combination with 2CdA counteracts growth of neoplastic mast cells and may thus represent a promising new candidate drug for the treatment of SM.

**0038**

**SENSITIZING LEUKEMIC CELLS TO GLUCOCORTICOSTEROIDS BY INHIBITING NFkB ACTIVATION**


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Glucocorticoids (GC) are commonly used in childhood leukemia, and induce apoptosis in GC-sensitive leukemic cells. Resistance to GC is a major adverse prognostic factor, occurring in ±20% of newly diagnosed childhood acute lymphoblastic leukemia (ALL) and in >50% of relapsed ALL, while acute myeloid leukemia (AML) is largely unresponsive to GC. Nuclear factor -kappaB (NFkB) is a transcription factor regulating the expression of cell survival genes, counteracting GC-induced cytotoxic effects both directly, via binding to the glucocorticoid receptor (GR) and indirectly. Blocking NFkB might prove a successful strategy to increase GC-sensitivity of leukemic cells. Chronic exposure to GC decreases IC50 of C2C7 t cell leukemia cells to dexamethasone (DEX) by 3-fold and that this effect of NFkB-acti- vation, sensitized these primary sensitive cells even 10-20 fold further for GC (Van der Heijden et al., 2004). Two myeloid leukemia cell lines, THP1 and U937, with inherent resistance to GC (IC50 for dexamethasone >10 μM) were also markedly sensitized for GC upon long-term exposure to SSZ (IC50 <0.1 μM). In SSZ-exposed cells GR, NFkB p65 and IkB protein expression was markedly increased. Expression of NFkB p50 remained unaltered, suggesting an inactive state of NFkB, which was confirmed by an NFkB activity assay. mRNA levels of all tested genes remained unchanged, suggesting that the GC-sensitizing effect is due to diminished post-transcriptional protein degradation. Consistently, co- incubation with a proteasome inhibitor further enhanced (5-fold) GC sensitivity in SSZ-exposed cells. To determine whether GC sensitization could also be achieved in cells that became GC-resistant due to previous GC exposure, we tested the GC sensitive CEM cell-line C7H2 and six CEM-C1A2 sublines with acquired GC resistance after GC exposure. All cells were exposed to 10 μM to 6 M) remained resistant (IC50>6 M). Two cell-lines only showed a transient increase in GC sensitivity. We are currently measuring the potential GC sensitizing effects of the proteasome inhibitor Bortezomib since it has been shown that this drug inhibits degradation of IkB. In addition, we have found earlier that co-incubation with Bortezomib further enhanced (5-fold) GC sensitivity in SSZ-exposed cells. These experiments will be per-
formed both in cell lines and primary patient samples. In conclusion, sever-

eral GC-resistant cell lines, both of lymphoid or myeloid origin, could be sensitized to GC by NFκB inhibitor sulfasalazine. SSZ could also
diminish GC resistance that was acquired after GC exposure, an event
often seen in the clinic. Further investigations are warranted to establish
whether therapeutic strategies targeting NFκB could be exploited to
(re)sensitize (relapsed) childhood ALL or even AML for GC.

**0039**

**RAPAMYCIN OVERCOMES DEXAMETHASONE RESISTANCE OF MALIGNANT PLASMA CELLS**

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**Background.** In multiple myeloma, several promising new agents with
mechanisms of action different from standard chemotherapy have been
developed during the past few years. These agents may be able to
improve the outcome of multiple myeloma, especially if they are able to
enhance the response towards conventional therapeutics when given
in combination. Aims. In order to provide rationale for combination
therapies, we performed *in vitro* studies on the effect of combinations of
conventional and novel drugs on human multiple myeloma cell lines.

**Methods.** Two cell lines, JK-6L and L363, were incubated with various
concentrations of drugs, alone or in combination, in the presence or
absence of human bone marrow stromal cells (BMSC). Cell growth was
measured in an MTS assay and results were evaluated for synergism.

**Results.** Inverse correlation on the effect of combinations of
Bcr-Abl, MDR1 and apoptotic proteins were detected by RT-
PCR and western blotting. Caspase-3 activity was determined using the
caspase-3 colorimetric assay. Mitochondrial membrane potential (MMP)
was measured using a JC-1 MMP detection kit. Cell cycle profiles of
cells were analyzed by flow cytometry. **Results.** K562/IMA-0.2, K562/Meg-01/IMA-0.2 and Meg-01/IMA-1 expressed about 2.3, 19, 2- and 5-fold resistance to imatinib, as compared to their parental
counterparts. There were an increased expression of Bcr-Abl, MDR1, Bcl-
2, and Bcl-XL and decreased expression of Bax protein in resistant cells as
compared to their parental counterparts. A decrease in caspase-3 activ-
ity and an increase in MMP was detected in resistant cells compared to
parental cells. Exposure to 500 nM IMA for 48 hr resulted in apoptosis
in about 75% and 60% of the population in K562 and Meg-01 sensitive
cells, while there were no apoptosis in K562/IMA-0.2 and only 20% of
apoptosis in Meg-01/IMA-0.2 cells. **Summary/Conclusions.** Various diverse
mechanisms have been reported for their involvement in the multidrug
resistance. In this study, it has been well documented that the degree of
BCR/ABL expression appears to be directly proportional to the levels of
imatinib resistance. In addition, there have been BCR/ABL-independent
mechanisms reported for deriving resistance against imatinib. Our
results revealed that besides Bcr-Abl overexpression, imatinib resistance
also depends on the inhibition of apoptosis as a result of up-regulation
of anti-apoptotic Bcl-2 and Bcl-XL proteins, down-regulation of pro-
apoptotic Bax protein, decreased caspase-3 activity, and increased MMP
K562/ or Meg-01/IMA-0.2 and Meg-01/IMA-1 cells.

**0041**

**IDENTIFICATION AND CHARACTERIZATION OF A HOMO-DIMER OF ABCG2 IN MATURE HUMAN ERYTHROCYTES**

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**Background.** Human ATP-binding cassette G2 (ABCg2, also known as
breast cancer resistance protein, mitoxantrone resistant protein, and
ABC placenta) is a member of the ATP-dependent binding cassette
(ABC) family of transporters. Similar to other well characterized ABC
transporters that are expressed in humans, namely P-glycoprotein (P-gp)
and Multi-Drug Resistance Protein 1 (MRP1), ABCG2 has been shown
to transport xenobiotics and other normal cell metabolites and anti-can-
cer drugs. ABCG2 is termed a half-transporter as two 70-kDa halves are
necessary to form a fully active transporter, although recent evidence
suggests that the protein may behave as multiple homo-dimers togeth-
er. i.e. as a homo-tetramer. ABCG2 has also been implicated in the trans-
port of hematopoietic stem cells and expression of P-gp in human 
pro-apoptotic Bcr-Abl, MDR1 and apoptotic proteins were detected by RT-
PCR and western blotting. Caspase-3 activity was determined using the
caspase-3 colorimetric assay. Mitochondrial membrane potential (MMP)
was measured using a JC-1 MMP detection kit. Cell cycle profiles of
cells were analyzed by flow cytometry. **Results.** K562/IMA-0.2, K562/Meg-01/IMA-0.2 and Meg-01/IMA-1 expressed about 2.3, 19, 2- and 5-fold resistance to imatinib, as compared to their parental
counterparts. There were an increased expression of Bcr-Abl, MDR1, Bcl-
2, and Bcl-XL and decreased expression of Bax protein in resistant cells as
compared to their parental counterparts. A decrease in caspase-3 activ-
ity and an increase in MMP was detected in resistant cells compared to
parental cells. Exposure to 500 nM IMA for 48 hr resulted in apoptosis
in about 75% and 60% of the population in K562 and Meg-01 sensitive
cells, while there were no apoptosis in K562/IMA-0.2 and only 20% of
apoptosis in Meg-01/IMA-0.2 cells. **Summary/Conclusions.** Various diverse
mechanisms have been reported for their involvement in the multidrug
resistance. In this study, it has been well documented that the degree of
BCR/ABL expression appears to be directly proportional to the levels of
imatinib resistance. In addition, there have been BCR/ABL-independent
mechanisms reported for deriving resistance against imatinib. Our
results revealed that besides Bcr-Abl overexpression, imatinib resistance
also depends on the inhibition of apoptosis as a result of up-regulation
of anti-apoptotic Bcl-2 and Bcl-XL proteins, down-regulation of pro-
apoptotic Bax protein, decreased caspase-3 activity, and increased MMP
K562/ or Meg-01/IMA-0.2 and Meg-01/IMA-1 cells.

**0040**

**MULTIDRUG RESISTANCE MECHANISMS IN HUMAN CHRONIC MYELOID LEUKEMIA CELLS**

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**Background.** Chronic myeloid leukemia (CML) is diagnosed by finding
a specific translocation between chromosomes 9 and 22. The result-
ing Bcr-Abl codes for a fusion protein with tyrosine kinase activity lead-
ing to uncontrolled cell growth. Imatinib, a Bcr-Abl inhibitor, induces
apoptosis in CML cells by stabilizing the non-ATP-binding form of Bcr-
Abl, and in turn, phosphorylation of its substrates. Aims. Despite the excellent clinical results with imatinib in CML, most patients have min-
imal residual disease and others will develop resistance which may even-
tually progress. In this study, the mechanisms responsible for imatinib resistance in human CML cells were investigated. **Methods.** The Ph³; human K562 and Meg-01; cells were exposed to step-wise increasing concentrations of imatinib. Subpopulations of cells that were able to grow in the presence of 0.2 and 1 µM imatinib, were then selected, and referred to as K562/ or Meg-
0042 FUNCTIONAL AND GENOME-WIDE ANALYSIS OF ACQUIRED RESISTANCE TO TRAIL/APO2L MEDIATED APOPTOSIS OF HL60 LEUKEMIA CELLS

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Background. Acute leukemia comprises malignant diseases of clonal character, to which specific treatment remains limited. Apoptosis induced by death receptor activation (i.e. by tumor necrosis factor-related apoptosis inducing ligand, TRAIL/APO2L) is a potential anti-tumor therapeutic mechanism. TRAIL, a member of the TNF family of death ligands, appears to specifically and efficiently kill tumor cells of diverse origin while sparing normal tissues. The TRAIL receptor family consists of five receptors: two death receptors (DR4/TRAIL-R1, DR5/TRAIL-R2), two decoy receptors (DR1/TRAIL-R3, DR2/TRAIL-R4), and osteoprotegerin (OPG).

Aims. Functional analysis of individual TRAIL receptors in HL60 myeloid leukemia cells and analysis of the molecular basis of TRAIL resistance.

Materials and Methods. TRAIL-resistant cells were selected from the original HL60 population using pressure of recombinant His-tagged TRAIL (200-2000 ng/ml). The expression of TRAIL receptors and CD14 were analyzed by flow cytometry using fluorochrome labeled antibodies and/or by real-time RT-PCR. Percentage of apoptotic cells was measured by flow cytometry using Annexin-V-FITC/Propidium iodide apoptosis detection kit. The contribution of individual TRAIL receptors on the transmission of apoptotic signal was measured using blocking antibodies to TRAIL receptors. The TRAIL resistance related genome aberrations were analyzed by genome-wide loss of heterozygosity (LOH) screening with marker density of 10cM and comparative genomic hybridization (CGH) assay. Results. The blockage of DR4 receptor significantly reduced the number of apoptotic HL60 cells compared to untreated controls. The blockage of DR5 receptor also inhibited TRAIL-induced cell death but the results did not reach statistical significance. Combination of anti-DR4 and anti-DR5 antibodies almost completely abrogated TRAIL-induced HL60 cell death and significantly reduced apoptosis compared to control or anti-DR4 antibody alone (p<0.01). Blocking of decoy receptors (DR1, DR2) or OPG of HL60 TRAIL-sensitive and TRAIL-resistant cell lines did not significantly affect the apoptotic signaling. Two distinct HL60 TRAIL-resistant phenotypes were identified based on the expression of TRAIL-receptors and CD14. Phenotype-1 (n=4) was characterized by the decreased expression of TRAIL receptors DR4, DR5, DR1, and DR2, CD14 and unchanged expression of OPG as compared to control TRAIL-sensitive HL60 cells. Phenotype-2 (n=3) was characterized by the decreased expression of DR5 receptor, increased expression of CD14, and undetectable expression of OPG compared to control TRAIL-sensitive HL60 cells. Using LOH assay we identified two genotypes. The first exhibiting deletion on the short arm of chromosome 1p22 and monosomy of chromosome 18, and the second had deletions/uniparental disomy on the short arm of chromosomes 2, 3, 6, and 14. The identified genotypes corresponded to TRAIL-resistant phenotype-1 and phenotype-2, respectively. CGH assay confirmed the loss of genomic material of whole chromosome 18. Further, the CGH detected a gain of genomic material at 1q21-23 of TRAIL-resistant phenotype-1 while the phenotype-2 cells did not show genomic defects of chromosome 1. Summary/Conclusions: In HL60 cells TRAIL-specific apoptotic signal is transduced predominantly through TRAIL receptor DR4. Decoy receptors, including OPG, did not play a role in TRAIL resistance. The identified TRAIL-resistant phenotypes are associated with distinct genomic conditions. Supported by: IGA MZ NR3317-4 and GAUK 50/2004/C.

0043 THE ADMINISTRATION OF REPEATED DOSES OF CYCLOPHOSPHAMIDE, INDUCES CYTOCHROME P450 IN RAT

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Background. Cyclophosphamide is used in high doses as a part of the conditioning regimen prior to stem cell transplantation. It is usually given for two or four consecutive days, primarily to facilitate engraftment of donor cells. Cyclophosphamide is activated in the liver by a 4-hydroxylation reaction catalyzed by cytochrome P450 (CYP) enzymes. Several studies have shown that cyclophosphamide induces its own metabolism, which affects its pharmacokinetics and pharmacodynamics after repeated doses. Aim. In the present study, we aimed to investigate the effect of repeated doses of cyclophosphamide on the CYPs in rat. The levels of mRNA, protein, and enzyme activity were investigated. Methods. Male Wistar rats were given 4 consecutive doses of CPA (2 dose levels). Plasma and livers were collected to study the pharmacokinetics of cyclophosphamide in plasma and to measure the levels of mRNA (by real time PCR), protein (by western blot) and enzyme activity (by microsomal incubation with cyclophosphamide) of CYPs, respectively. Results. mRNAs of CYP2B1 and 2B2 were significantly induced with repeated dosing. Protein levels were also induced and autoinduction of CPA metabolism to 4-hydroxylation was found. Conclusion. Repeated dosing of CPA leads to autoinduction of CPA metabolism and induction of CYP2B1 mRNA and protein in rat. This knowledge may help in optimizing the dosing regime of cyclophosphamide in patients to keep plasma levels within the therapeutic range. It may also help in minimizing drug-drug interactions and hence increase the therapeutic efficacy and reduce side effects of cyclophosphamide in cancer patients.

0044 OPTIMIZATION OF THERAPY FOR THIOPURINE S-METHYLTRANSFERASE DEFICIENT CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS

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Background. Thiopurine S-methyltransferase (TPMT) is an enzyme that catalyzes the demethylation of thiopurine drugs, such as 6-mercaptopurine (6-MP) and thioguanine, commonly used in therapy for childhood acute lymphoblastic leukemia (ALL). In BFM protocol for childhood ALL, 6-MP is administered during maintenance therapy. Patients with low TPMT activity experience severe hematological toxicity when standard 6-MP doses are used. It is now well established that lower TPMT activity can be due to TPMT gene mutations. Three alleles account for more than 95% of the clinically relevant TPMT variants: TPMT*2, TPMT*3A and TPMT*3C. Wild type has been designed as TPMT*1. TPMT*2 allele contains single G238C mutation, TPMT*3C-A719G mutation, TPMT*3B-G460A mutation and TPMT*3A allele has two mutations (G460A and A719G). Aim. The purpose of this study was to determine the relevance of TPMT gene mutations in the management of childhood acute lymphoblastic leukemia (ALL). Methods. Blood samples from 100 children with ALL were analyzed for TPMT mutations, using polymerase chain reaction-based assays (PCR-RFLP and ARMS). For 50 patients TPMT variant alleles were determined retrospectively, after completing the standard BFM protocol maintenance therapy. Maintenance therapy period was compared according to patients' TPMT genotypes. For the other 50 patients TPMT variant alleles where determined prospectively. For prospectively detected patients with TPMT variant alleles we introduced therapy protocol modification in a way that if leucopenia was noticed, only the dose of 6-MP was reduced but there were no reduction of methotrexate (MTX) doses. The number of weeks when full, reduced dose or no 6-MP therapy was given, was determined for each patient during the maintenance therapy. Number of neutropenic fever was also considered as a toxic effect of 6-MP therapy. Results. Of 100 patients participating in this study, 89% were homozygous for TPMT*1 (W/W), 9% were heterozygous for TPMT*1/*3B and 2% were homozygous for TPMT*1/*2. One patient was double heterozygous for TPMT*3A/*3B.
(M/M). Among 50 patients retrospectively analyzed for TPMT variants 6 were found to be W/M. Mean duration of full dose therapy was signifi-
cantly longer (p<0.01) in W/M patients (54 weeks) than in W/M (87.5 weeks).
Mean duration of period without therapy was significantly longer (p<0.01) in W/M patients (11.3 weeks) than in W/M (8.4 weeks).
Neutropenic fever occurred in all of the patients (1-4 times). For four prospectively detected W/M patients, therapy protocol was modified (dosage reduction of 6-MP by 25-50%). In contrast to W/M patients retrospectively analyzed, these W/M patients neither missed the therapy nor developed febrile neutropenia. Conclusion. The ability to tolerate 6-
MP based maintenance therapy was used as a surrogate marker of hema-
tological toxicity in childhood ALL. We found that even patients het-
erozygous for TPMT variant alleles are at greater risk of thiopurine drug-
related leucopenia. Lowering doses of 6-MP in heterozygous TPMT deficient patients while allowing administration of full dose of MTX, might be an optimal way of treatment for this group of patients. These results justify performing TPMT genotyping before initiating thiopurine therapy in all children diagnosed with acute leukemia to minimize con-
sequent toxicity.

0046
IMATINIB AND HUMAN ORGANIC CATION TRANSPORTER 1 (hOCT1): CHARACTERISATION OF TRANSPORT IN STABLY TRANSFECTED MYELOID CELLS
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Imatinib is an important drug for treating chronic myeloid leukaemia (CML). However, not all patients achieve a major cytogenetic response (MCR). Recent studies suggest that hOCT1 expression is a determinant of response to imatinib therapy in the cellular uptake and efflux of imatinib, and particularly whether this may influence clinical outcome. A previous study in our lab showed that the level of expression of hOCT1 is modified in a MDR1 wt cell line to a MCR, with or without induction therapy. Here we report on the expression and functional evaluation of hOCT1 in tumor cell lines, using a number of clones with different expression levels. These clones were then used for transport studies, in order to determine hOCT1 expression in determining the outcome of imatinib treatment in CML.

0047
CHARACTERIZATION OF THREE CASES OF ANEMIA/MENTAL RETARDATION SYNDROMES (ATR-16) IN THE NETHERLANDS, USING MULTIPLEX LIQUID-DEPENDENT PROBE AMPLIFICATION (MLPA)
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Background. Two distinct and rare syndromes of a-thalassemia asso-
ciated with mental retardation are known to date. One is characterized by the occurrence of large deletions involving the α-globin gene cluster on chromosome 16p (ATR-16 syndrome) and is most likely a continu-
ous variant of the S syndrome. The other (ATR-16X) involves loss of the X-
linked XNP gene, coding for helicase-2, a putative global transcriptional
regulator. At present the molecular tests commonly used to identify deletion types of a-thalassemia and ATR-16 are gap-PCR, Southern blot or Fluorescent in situ Hybridization (FISH) analysis. However, the appli-
cability of these techniques is limited to known deletions, may involve radio-activity, is dependent upon the hybridization probes available and may require time consuming and laborious cell culture to generate metaphase chromosome spreads. *Aim.* Clinical description and molecular characterization of three independent patients presenting with a microcytic hypochromic anemia at normal ferritin levels and mild mental retardation. *Methods.* Ligation Dependent Probe Amplification (MLPA) is used to identify (unknown) deletions causing α-thalassemia, which remain undetected by the common techniques presently used in molecular diagnostics. *Results.* We have developed a subset of MLPA probes spread along a region of approximately 2Mb from the telomere of chromosome 16 up to the PDK-gene for high resolution mapping of rearrangements causing α-thalassemia. One Dutch Caucasian female (50 yrs) was identified because of a persistent microcytic hypochromic anemia at normal ferritin levels, positive I.B. test and no abnormalities at the molecular level using the standard detection *Methods.* The other two patients were identified because of (mild) mental retardation and the detection of a subtelomeric deletion by MAPH and mapped in detail by MLPA in the present study. The deletions causing ATR-16 in these patients vary in length between 1.5 to 1.9 Mb. *Conclusion.* We have developed a rapid and simple technique based on Multiplex Ligation-Dependent Probe Amplification for high resolution mapping of rearrangements involving the tip of the short arm of chromosome 16. Three cases show the rare E6 syndrome, two of which were found by screening for subtelomeric imbalances by FISH or MLPA and not by hematological analysis. This would plead for more alertness when a patient presents with mild to moderate MR and microcytic hypochromic anemia with normal ferritin levels as suggestive for ATR-16.

**0048**

**INDIVIDUAL PATIENT DATA ANALYSIS IN YOUNGER ADULTS WITH NORMAL KARYOTYPE AML: DIFFERENTIAL EFFECTS OF MOLECULAR MARKERS ON CLINICAL OUTCOME RESULTS OF THE AML STUDY GROUP**

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Background. Mutations in the genes encoding NPM1, FLT3 (FLT3 ITD, FLT3 TKD), CEBPA, MLL (PTD) and NRAS have been identified as molecular markers in acute myeloid leukemia (AML) exhibiting a normal karyotype. Approximately 70% of normal karyotype AML have mutation in one of these genes. In most of the recent studies univariate analyses of outcome measures were performed for singles markers not taking into account potential interactions. Furthermore, little is known about the differential effect of different postremission therapies in the various genotypic subsets. *Aims.* To evaluate the prognostic impact of NPM1, FLT3 (FLT3 ITD, FLT3 TKD), CEBPA, MLL (PTD) and NRAS gene mutations on response to induction therapy and on survival probabilities following different postremission strategies [high-dose cytarabine-based chemotherapy (chemo), autologous (auto-SCT), or allogeneic stem cell transplantation (allo-SCT)]. *Methods.* Patients [16 to 60 years of age] were entered on four AMLSG treatment trials [AML-2/95, AML-1/99, AML HD95, AML HD98A]. Patients received two cycles of induction therapy with standard-dose cytarabine combined with etoposide and idarubicin. After a first consolidation therapy, in all four trials patients were assigned to allo-SCT if an HLA-identical sibling donor was available; in the AML-2/95 and AML HD93 trials, all other patients were assigned to chemo, whereas in the AML-1/99 and AML HD98A trials patients were randomized between chemo and auto-SCT. Diagnostics leukemia specimens were analyzed for mutations in the above genes. *Results.* In 16 and 2004, 872 patients exhibiting a normal karyotype were registered. Results of the mutation status were as follows (total number of samples analyzed; incidence of mutations): NPM1 (n=526; 53%), FLT3-ITD (n=516; 31%), FLT3-TKD (n=602; 11%), CEBPA (n=492; 16%), MLL-PTD (640; 7.3%), and NRAS (505; 13%). Complete remission rate (CR) was 77%. A logistic regression model identified the NPM1+/-FLT3-ITD (p<0.001) and the CEBPA+ genotype (p=0.05) as favorable prognostic factors for CR achievement. Cox proportional hazard models with limited backward selection for RFS and OS revealed age <48 years (hazard ratio (HR) 0.69 and 0.61), availability of an HLA-identical family donor (HR 0.58 and 0.73), the CEBPA+ (HR 0.42 and 0.36) and the NPM1+/-FLT3-ITD (HR 0.34 and 0.42) genotype as significant prognostic factors. There was no benefit for auto-SCT in any of the subgroups. The prognostic impact of an HLA-identical family donor varied significantly among the different molecular subgroups. *Conclusions.* Specific genotypes emerge as highly significant factors for response to induction therapy and for survival in patients with normal karyotype.

The value of allo-SCT needs to be revisited in the various genotype subsets.

**0049**

**DEVELOPMENT OF AN INTEGRATED ASSAY FOR QUANTITATIVE REAL TIME DETECTION OF BCR-ABL RNA FROM PERIPHERAL BLOOD**

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*Background.* T-PCR testing for BCR-ABL is done today primarily using laboratory developed assays. While each of these assays may be consistent within any particular lab, there is still lack of complete consensus on assay design, results reporting, and reference ranges. Our goal was to develop a highly integrated assay that could be run easily by a laboratory technician without any special training in molecular techniques, and would give a standardized answer at any laboratory.

*Methods.* The Cepheid Xpert® BCR-ABL Monitor assay is designed to co-amplify the BCR-ABL transcript and the ABL transcript (the endogenous control). The assay only requires a few simple manual pipetting steps.
steps, followed by fully automated nucleic acid purification, nested RT-PCR, and data analysis. A 200 ul aliquot of whole blood is mixed with proteinase K and lysis reagent to inactivate nucleases and release the nucleic acid from the cells. After addition of 1 ml of ethanol to the lysed sample, the mixture is added to the test cartridge using a transfer pipette. Wash, rinse, and elution reagents are also added to designated ports in the test cartridge, the lid is closed, and the cartridge is loaded into the GeneXpert®. By moving the sample and reagents into different chambers in the cartridge during the test process, the GeneXpert® (1) isolates the total RNA from lysed whole blood by binding the RNA to the solid phase purification material, (2) washes and rinses away inhibitors, (3) elutes the RNA, (4) hydrates the reagent beads and combines the eluted DNA to form cDNA, (5) moves the sample and reagent mixture into the reaction tube, (6) performs quality checks to ensure that reagent preparation was successful, (7) performs a one step RT-PCR followed by nested real-time PCR (8) reviews the signal from both the ABL endogenous control and the BCR-ABL transcript for acceptability, and (9) calculates the delta Ct between the two signals. The test process for BCR-ABL takes approximately 2 hours and 20 minutes.

Results and Conclusions. The specificity of the assay was tested using 42 citrate and EDTA bloods from normal individuals and a collection of 12 bloods from patients with other hematologic disorders including acute myelogenous leukemia, acute lymphocytic leukemia, Hodgkin’s lymphoma, non-Hodgkin’s lymphoma, and follicular lymphoma. All of these samples were negative for BCR-ABL, yielding a specificity of 100%. A collection of 46 samples from patients with CML were tested by the Xpert BCR-ABL Monitor assay and by a laboratory-developed RT-PCR reference assay. There was 85% agreement of negative results (17/20) and 100% agreement of positive results (26/26). A precision study indicated that the assay is highly reproducible between sites, days, instruments, and operators (Table 1).

0050
HARMONIZATION OF BCR-ABL TRANSCRIPT QUANTIFICATION USING AN UNIFORM CONTROL PLASMID IN 37 INTERNATIONAL LABORATORIES
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Background. Serial measurement of leukemia specific BCR-ABL transcripts is a valuable approach to monitoring individual patients with chronic myelogenous leukemia after therapy. However, heterogeneity of molecular approaches results in a lack of comparability between different studies. Thus, there is an unmet need for harmonization of both procedures and expression of Results. In a series of consensus meetings within the European LeukaemiaNet recommendations for achieving optimal sensitivity and standardization have been elaborated: (a) use of at least 10 ml peripheral blood processed within 36 hrs; (b) bedside RNA stabilization for multicenter trials; (c) standardized PCR protocols optimized for each platform; (d) use of a single plasmid containing target and housekeeping gene to avoid dilution effects; (e) use of beta-glucuronidase (GUS) as internal controls. Aim. The aim of the study was to assess the variability of results obtained from 37 different labs in 14 countries using the PAXGene Blood RNA Kit (PreAnalytiX, Hombrechtikon, Switzerland) for RNA extraction, individual protocols for cDNA synthesis, 5 different PCR platforms (TaqMan, TM, n=25, LightCycler, n=13, Roche, n=1), and optimized quantitative RT-PCR conditions. Methods. In order to standardize results, b3a2 BCR-ABL and GUS sequences were cloned into a plasmid (PCR TOPO vector (Invitrogen, Carlsbad, CA), which was distributed to all participants in serial dilutions as external control for quantification of BCR-ABL, total ABL, and GUS. Ten samples containing dilutions (10, 2, 1, 0.1%) of b3a2 or b2a2 BCR-ABL positive in normal leukocytes and negative controls were prepared, blinded, and shipped to the participants. Transcript numbers were determined in triplicates, ratios BCR-ABL/ABL and BCR-ABL/GUS were calculated and expressed in percent. Results. Median ratios BCR-ABL/ABL for b3a2 samples were 9.1, 1.8, 0.85, and 0.11%; for b2a2 samples 9.5, 1.6, 0.84, and 0.11%. Median ratios BCR-ABL/GUS for b3a2 samples were 3.4, 0.77, 0.37, and 0.042%; for b2a2 samples 2.8, 0.48, 0.29, and 0.081%. Forty of 57 participants (11%) detected low BCR-ABL copy numbers in negative control samples. The coefficients of variation (CV) for all participants, TM, and L. users were 0.62, 0.57, and 0.68 for ratios BCR-ABL/ABL and 1.05, 1.22, and 1.22 for ratios BCR-ABL/GUS, respectively. Standard errors to the regression line were significantly lower evaluating ratios BCR-ABL/GUS (median 0.075, range 0.0046-0.90) compared to ratios BCR-ABL/ABL (median 0.18, range 0.022-2.2, p<0.001). Overall, mean TM ratios were 1.7 times higher than LC ratios indicating a difference of the amplification efficiency. Conclusions. Harmonization of BCR-ABL mRNA quantification is feasible employing a common plasmid for BCR-ABL, total ABL, and GUS. However, the remaining variability of results indicates minor differences of the PCR efficiencies using individual protocols. We therefore suggest the use of a common standard plasmid, the introduction of a calibrator, and regular control rounds to achieve comparability of results between individual labs.

0051
THE IDENTIFICATION OF JAK2V617F IN PATIENTS WITH POLYCYTHEMIA IS HIGHLY CORRELATED WITH CONVENTIONAL CRITERIA FOR DIAGNOSIS OF POLYCYTHEMIA VERA
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Background. In 2005 it was recognised that different myeloproliferative disorders (MPD) share an activating JAK2 tyrosine kinase mutation (JAK2V617F). The frequency of the mutation varies being greatest in polycythemia vera (PV) (65-97%) and less common in essential thrombocythemia (ET) (2-9%) and idiopathic myelofibrosis. Aims. We wished to assess the utility of screening for the JAK2V617F mutation in patients with known or suspected myeloproliferative diseases. In particular whether screening can contribute to the diagnosis or even substitute for other investigations in polycythaemia and if so what would be the economic implications. Methods. We adapted the screening assay for JAK2V617F described by Baxter and over 6 months screened 146 consecutive patients with previously diagnosed or suspected MPD from different parts of New Zealand. Indications for screening were classified as polycythaemia, thrombocytois, myelofibrosis and other. We undertook a retrospective review of the case records for those 66 patients who had been screened for polycythaemia, to ascertain which investigations had been performed at presentation, their results and information on subsequent management. Results of these investigations were used to establish whether a diagnosis of PV, using WHO or PVSG criteria. The frequency and cost of the various investigations performed for all patients was calculated. Results. The JAK2V617F mutation was detected in 31 of 66 (47%) patients with polycythaemia, 34 of 67 (51%) patients with thrombocytosis and 5 of 10 (50%) patients with myelofibrosis. Other patients screened included a patient with multiple myeloma who received an allograft from a donor with JAK2V617F and a patient with MDS and thrombocytosis (wild type allele). Of the 66 patients with polycythaemia, 12 patients were excluded since they had insufficient elevation of red cell mass, haemoglobin or haematocrit to meet criteria for PV. Of the remaining 54 patients, 42 patients had been sufficiently investigated to either diagnose or exclude PV. 24 of 25 (96%) patients with PV and 17/17 patients with JAK2V617F were included had only the wild type allele. Twelve patients had been insufficiently investigated to determine whether or not they met criteria for PV: - of these 5 were JAK2V617F All patients with PV were receiving appropriate treatment with venesecion or myelosuppression. No patient in whom PV was excluded had received myelosuppressive therapy though many were on vesionese programmes. 10200 EUR was spent on investigating 54 patients with polycythaemia. If JAK2V617F mutation screening had been undertaken as the initial investigation for these patients, proceeding to further investigations only in those without JAK2V617F, the total cost of investigations would have been 12400 EUR. Summary/Conclusions. In our patients with sufficiently elevated haemoglobin to meet WHO criteria for PV, the identification of JAK2V617F is 94% sensitive and 100% specific for PV. The varying frequencies of JAK2V617F reported in different series of PV patients may be due in part to the inclusion of patients in the PV group who do not meet diagnostic criteria for PV. Early screening for JAK2V617F in patients with polycythaemia can give a prompt and unequivocal diagnosis of PV without significant additional cost.

Reference
Background. Monoclonal TCRβ+/CD4+/NKα+/CD8+dim T represent a subgroup of monoclonal LGL lymphoproliferative disorders different from both CD8+ T-LGL and NK-cell type LGL leukemias. The recently described TCRβ+/CD4+-T-LGL leukemia/lymphocytosis has been shown to be associated in around one third of cases with a neoplastic other than the T-LGL, which prompted us to hypothesize that the TCRβ+/CD4+-T-LGL may proliferate and expand as an effort of the immune system to control tumor growth, supporting in some way the antigen-driven selection model. We typed for HLA class I and II genes in patients with different TCR-β expansions. TCR clonotypes and VDJ rearrangement structure were analyzed in a cohort of patients with CD4+ T-LGL expansions. Aims. Analyse the possible association between the TCR β-family expanded with HLA and CD35 hypervariable region expressed in patients with β expansions LGL. TCR αβ+/CD4+/CD8+.neg. Methods. Total of 36 individuals (19 males and 17 females; mean age of 64±11 years, ranging from 40 to 81 years) having a TCRαβ+/CD4+/NKα+/CD8+dim monoclonal T-LGL lymphoproliferative disorder were studied. For the immunophenotypic studies a panel of 24 monoclonal antibodies (MAb) directed against an identical number of members of 21 different HLA or CD35 families was used. A genotype for HLA-ABC and both HLA-DRB1 and HLA-DQB1 was determined by SSO PCR. DNA were amplified and clonal products from the VH gene PCR were sequenced directly using the BigDye Terminator Cycle Sequencing Reaction Kit. Results. In all cases studied, expanded CD4+ LGL T-cells showed relatively high SSC features as compared to normal PB CD4+ T-lymphocytes and common phenotypic characteristics, consisting of TCRαβ+/CD8α+dim cells with a typical cytotoxic (granzyme B+, CD56+, CD57+, CD11b+) activated/memory T-cell immunophenotype (CD2−/−, CD7−/−, CD11a−/−, CD28−, CD62L−, HLA-DR+). Flow cytometric analysis of the TCR-β repertoire of CD4+/CD8α+dim LGL T-cells was consistent with a (mon)clonal expansion in all cases studied, which accounted for 75±26% of all PB CD4+ T-cells. In 27 cases the expanded TCR-β family was identified with the panel of TCR-β reagents used, corresponding to TCR-Vβ13.1 in 15 cases (42%), TCR-Vβ 2.1 in 2 (5.6%), TCR-Vβ 3.1 in 2 (5.6%), TCR-Vβ 8.1 and Vβ 8.2 in 2 (5.6%), TCR-Vβ17.1 in 2 (5.6%), TCR-Vβ22 in 2 (5.6%) and TCR-Vβ11 or TCR-Vβ14.1 in one case each (2.8%). In the remaining 9 patients, the expanded TCR β-family was not identified (25%) with the panel of MAb used. All 15 patients who showed expansions of TCR-Vβ 13.1+ CD4+ T-cells were HLA-DRB*0701+. Comparison of CD35 size distribution in clonal CD4+/CD8α+dim T-cells from the same patients showed a highly restricted usage of VHDJH segments and shared CDR3 configurations/sequences. These findings suggest that the expansions were selected for this unique TCR structure. These results strongly support that Vβ13.1 CD4+ T cells with the described CD35 motif may recognize a specific antigen presented by DR7 molecules, indicating the existence of a common associated antigen.

**0053**

**MDR1, MRP AND LRP EXPRESSION IN PATIENTS WITH UNTREATED ACUTE LEUKEMIA: CORRELATION WITH TC-99M MIBI BONE MARROW SCINTIGRAPHY**

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Background. Multidrug-resistance (MDR) phenotype concerns altered membrane transport that results in lower cell concentrations of cytotoxic drugs in many cancer types, including leukemia and is related to the overexpression of a variety of proteins that act as ATP dependent extrusion pumps. Tc-99m Sestamibi (MIBI) is a transport substrate for Pgp pump. Aim. We assessed the bone marrow uptake of Tc-99m MIBI and its correlation with messenger RNA (mRNA) levels of MDR1, Multidrug-Resistance Associated protein (MRP) and Lung Resistance Protein (LRP) in acute leukemia. A total of 26 patients with newly diagnosed acute leukemia (8 ALL and 18 ANLL) were included in the study. The expression of MDR1, MRP, and LRP on mRNA levels were assessed by semi quantitative RT-PCR (Roche Light Cycler System, Metis Biotechnology primers and probes for MDR1, MRP and LRP) in the blasts from the bone marrow samples. Planar images of the pelvis and thorax were acquired 20 min after injection of 740 MBq Tc-99m MIBI. The MBI uptake in the bone marrow was evaluated using a quantitative scoring system with determination of the tumour-to-background ratios for the bone marrow in areas that included the proximal femur, anterior iliac crest and sternum. The correlation between the RT-PCR results and MIBI uptakes was analysed by using Spearman’s rank correlation coefficients with two-tailed test of significance. Results. There was an inverse relationship between Tc-99m MIBI uptake of bone marrow and both mRNA levels of MDR1 and MRP (p = 0.000, r = - 0.735 and p = 0.001, r = - 0.610, respectively). No correlation was found between MIBI uptake and mRNA levels of LRP. Conclusion: Increased expression of MDR1 and MRP correlates with a low accumulation of Tc-99m MIBI in bone marrow areas in patients with acute leukemia. As a functional imaging, Tc-99m MIBI bone marrow scintigraphy can identify the MDR1 and MRP phenotype, but not LRP, in patients with acute leukemia.
Methods. Peripheral blood granulocytes were separated from altogether
151 patients with already diagnosed or suspected Ph+ MDP. Patients with polycythaemia vera (PV), secondary polycyloblasia (SP), essential thrombocytocemia (ET), idiopathic myelofibrosis (IMF) and unf diferentiated MDP (MPD-U) were included in the study. The cells were lyzed and RNA extracted using the Trizol reagent. Following reverse transcription, two methods were employed to detect JAK2 mutations. 1) The method of Baxter et al., using primers forward, using reverse primers reverse and amplifying them hybridizing to the mutated allele and a common reverse primer recognizing both the mutated and unmutated JAK2 alleles. Homo- and heterozygocity of the mutated gene was discriminated by sequencing analysis. 2) The allelic discrimination real-time RT-PCR assay that uses one pair of primers and two dual labeled TaqMan probes with LNA modified nucleotides. The probes differ at the polymorphic site, one of them is complementary to the wild-type JAK2 allele and the other to the mutated one. The result is given by the curves arising from measured fluorescence of two different reporter dyes during the real-time PCR (FigURE 1).

Results. Altogether 151 samples of patients with suspected Ph-
MDP were analyzed using both of the above mentioned methods for
JAK2 detection. In both of the assays, the same result was obtained,
JAK2 mutation being found in the same 71 out of 151 patients (47.0%). Ten of the 71 JAK2 mutations (14.1%) were homozygous, half of which were found in PV patients. In ET, JAK2 mutations were demonstrated in 22/57 (38.6%) patients, none of them was homozygous. Of 48 patients with PV, 35 had mutations (76.7%), whereas only 7/10 patients with SP had the mutated allele of JAK2 gene. Six of 20 (50.0%) individu-
als with IMF had JAK2 mutations (3 were homozygous). In the remain-
ing 21 MPD-U patients, 9 mutations (42.9%) were detected. Conclusions. The TaqMan allelic discrimination assay yields the same results as the method of Baxter et al. In contrast to the latter, it is very simple and does not require sequencing to distinguish between homo- and heterozy-
gotes. Thus it is less laborious and time-consuming and therefore also suitable for routine clinical laboratory testing.

References

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0056

HIGH FREQUENCY OF AML1 MUTATIONS IN BOTH DE NOVO MYELODYSPLASTIC SYNDROME AND CHRONIC MYELOMONOCYTIC LEUKEMIA BUT WITH DIFFERENT MUTATION PATTERNS

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Background. Transcription factor AML1 is essential for normal hematopoiesis. AML1 mutations have been found in therapy-related myelodysplastic syndrome (MDS) but were rarely described in patients with de novo MDS or chronic myelomonocytic leukemia (CMML). Aims. We sought to determine the frequency and patterns of AML1 mutations in de novo MDS and CMML and to correlate the mutation status with the clinicohematologic features. Methods. Mutation analysis of AML1 was performed on bone marrow samples from 76 patients with MDS (11 RCDM, 31 RAEB1 and 34 RAEB2) and 67 patients with CMML by direct sequencing for all RT-PCR products amplified with 3 overlapping primer pairs which cover the coding sequences of AML1 gene from exon 3 through exon 8. Results. At initial presentation of MDS, 14 of 76 MDS patients (18.4%) had AML1 mutations; 3 mutations were located in Runt homology domain (RHD) (exons 3-5) whereas 11 mutations were located in the non-RHD region (exons 6-8). The 14 AML1 muta-
tions included 6 missense mutations, 4 nonsense mutations, 2 frameshift mutations, and 2 silent mutations. AML1 mutations were detected in 27 of 67 CMML patients (40%) at initial diagnosis, 17 patients had 19 mutations in RHD and 10 patients had mutations located in the non-RHD region; the patterns of 29 mutations consisted of 7 missense muta-
tions, 5 nonsense mutations, 14 frameshift mutations and 3 silent muta-
tions. One CMML patient had two missense mutations in RHD, another
patient had two frameshift mutations in RHD. Cloning analysis showed that the two mutations were on different alleles in both patients. The frequency of AML1 mutations was significantly higher (p=0.005) in CMML than in MDS (p=0.020). CMML patients had a higher frequency of frameshift mutations as compared with MDS patients (p=0.045). AML1+ CMML patients had a significantly lower platelet count than AML1– patients (p=0.025). There were no differences in age, sex, hemoglobin level, WBC count, percentages of blasts in bone marrow and peripheral blood, morphologic subtype, and cytogenetic risk group between AML1+ and AML1– patients in CMML or MDS. Eleven of 14 AML1+ MDS patients (78.6%) progressed to AML compared with 39 of 62 AML1– patients (62.9%) (p=0.357). Eleven of 27 AML1+ CMML patients (40.7%) progressed to AML compared to 13 of 40 AML1– patients (32.5%) (p=0.605). Time to AML transformation and overall survival of AML1+ patients did not differ from AML1– patients in both MDS and CMML groups. Conclusions. Our study showed that AML1 mutations were frequently detected in de novo MDS and CMML, especially the latter. Patients with CMML were more frequently associated with mutations in RHD and frameshift patterns compared to patients with de novo MDS.
ACCURATE V617F JANUS KINASE 2 MUTATION GENOTYPING COMBINING ARMS PCR AND CAPILLARY ELECTROPHORESIS

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Background. During the last decade CML has paved the way for tyrosine kinase-targeted therapy. Recently, several groups have demonstrated the pivotal role of the acquired V617F Janus kinase 2 (JAK2) mutation in Ph+ Chronic Myeloproliferative Disorders, indicating a novel potential therapeutical target in CMPDs. Although this mutation has been described in the major CMPDs subtypes, controversy still remains about its exact prevalence in each subcategory. This could partially indicate the difficulties of achieving a precise diagnosis relying on the current WHO and FVSG diagnosis criteria. On the other hand, different technologies characterized by various sensitivity and sensibility have been used in the present studies, highlighting the need for standardized, accurate and sensitive assays. Aims. We describe here a new V617F JAK2 mutation screening approach. The analytical properties of this assay are described and compared with the traditional PCR sequencing strategy, site-specific restriction analysis and ARMS PCR followed by slab gel electrophoresis. Finally, we report our experience in genotyping 20 controls and 222 patients sent to our lab for typical and atypical MPD diagnosis. Clinical parameters, as well as previously described clonality assay, i.e. granulocytes PRV-1 expression, were evaluated in regard of the JAK2 V617F genotype. Methods. We developed a new V617F JAK2 mutation screening assay which combined the previously described amplification refractory mutation system (ARMS) and capillary electrophoresis performed with the Agilent 2100 Bioanalyzer apparatus (ARMS-cap). Serial dilutions of 100% V617F-homozygous HEL cell line into non mutated Jurkat cell line were assessed by ARMS PCR, ARMS-cap, PCR sequencing and BsaXI site-specific restriction analysis. The sensitivities of these tools were compared.

Results. ARMS PCR followed by slab-gel electrophoresis presented a JAK2 V617F detection sensitivity ranging from 1/32 (DNA) to 1/256 (RNA), which is far better than the PCR sequencing resolution (1/8 to 1/16). BsaXI cleavage improved the sensitivity when starting from DNA (1/64-1/128) whereas its best sensitivity level was the same as ARMS RNA PCR (1/256). ARMS-cap, offering a resolution of 1/64 (DNA) and 1/512 (RNA), improved the sequencing approach as well as the ARMS PCR followed by slab-gel electrophoresis. These results were in the same range than the BsaXI cleavage assay (1/128-1/256). Using this tool to assess our cohort of patients, we found the following incidence of JAK2 V617F mutation: PV, 90% (18/20); TE, 44% (12/27); IME, 80% (4/5) and aCMMD, 24% (16/66). None of the 10 ALL, 10 de novo AML, 14 primary AML, 10 NHL, 10 HES/CEL, 25 MDS, 11 CMML were found to be mutated whereas we found 1 CNL and 4% MDS (1/25) harboured the mutation. Conclusions. JAK2 ARMS PCR assay combined with capillary electrophoresis represents a new and sensitive assay for an accurate V617F JAK2 mutation screening. The analytical properties of this assay overcome the classical PCR-sequencing or ARMS PCR followed by slab-gel electrophoresis approaches. Whereas this approach only slightly improve the raw sensitivity level achieve by BsaXI site-specific restriction analysis and recording offers an objective and reproducible tool for V617F JAK2 mutation screening.
0059 SENSITIVE DETECTION OF C-KIT POINT MUTATIONS IN PATIENTS WITH MASTOCYTOSIS BY D-HPLC

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Background. The majority of patients with systemic mastocytosis (SM) are associated with an activating mutation in codon 816 of c-kit (CD117), a tyrosine kinase receptor on the surface of mast cells. This abnormality is regarded as being causative for the pathogenesis of the disease and is a potential target for therapeutic intervention. The sensitivity of screening procedures for mutations by direct sequencing might be compromised by a small proportion of malignant cells in the bone marrow (BM) sample. Therefore, sensitive methods are required for diagnosis and surveillance of pts during therapy. Imatinib inhibits the c-KIT tyrosine kinase at pharmacological doses with an IC50 of 0.1 µM, but it does not affect D816V mutants. Recent in vitro data suggest that both dasatinib, nilotinib (AMN107) and midostaurin (PKC412) have inhibitory effects on c-KIT D816V mutant cells. Aim. We sought to set up a sensitive strategy to detect c-kit mutations in BM and peripheral blood (PB) samples using D-HPLC (denaturing-high performance liquid chromatography) and/or direct sequencing of PCR fragments spanning the promoter region, all the exons and the intron-exon boundaries. In this study, D-HPLC was optimized to detect down to 0.1-0.5% HMC-1 cells in a background of NB4 cells harboring wildtype c-kit. The technique was then applied to 79 pts fulfilling the WHO criteria for SM. In case of a positive D-HPLC signal, c-kit exon 17 was sequenced to confirm the mutation using D-HPLC eluates and/or cDNA from the original sample.

Results. D-HPLC was optimized to detect down to 0.1-0.5% HMC-1 cells. In comparison, the detection limit for D816V point mutations by conventional sequencing was 10%. BM (n=79) and PB (n=7) samples from 77 pts (42 m, 35 f) have been investigated. Median age was 51 yrs (range 28-84). At diagnosis, D-HPLC was positive in 67 BM (80.5%) and 79 PB cases (99%). Conventional sequencing revealed the D816V mutation in 56 pts, one pt was positive for the D816H mutation. In addition to D816V, an I798I polymorphism was observed in one pt. The analysis of PB only revealed D-HPLC positivity in 5/7 pts with a consecutive detection of a D816V mutation in three pts. Conclusions. (i) D-HPLC combined with conventional sequencing is a reliable and sensitive method to detect c-kit mutations in the majority of pts with BM. (ii) The method is eligible for the surveillance of pts during therapy with novel tyrosine kinase inhibitors.

0060 MUTATIONAL SCREENING IN A POPULATION OF HAEMOPHILLA A SUBJECTS FOLLOWED AT THE HAEMOPHILIA CENTRE IN HOSPITALAR DE COIMBRA

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Introduction. Haemophilia A (HA) is an X-linked hemorrhagic disorder associated with blood coagulation factor VIII (FVIII) deficiency. FVIII gene mutations type are closely correlated with the FVIII activity levels, however, the same mutation can generate different phenotypes and not all the haemophilia patients with FVIII levels <1% bleed with the same intensity and frequency. Aim. In order to provide the HA carriers identification and eventual prenatal diagnosis, we performed the FVIII gene molecular studies in the HA patients followed at our Haemophilia Centre. In this study we present the mutations found, their correlation with the severity of the disease and the development of FVIII inhibitors. In the severe haemophiliaics group we screened the Factor V Leiden (FVL) and prothrombin G20210A mutation developed inhibitors and is a low responder. Four severe HA patients developed FVIII inhibitors; two, high responders, carry the IVS22 inversion (2/14), 1 have the IVS1 inversion (2/3) and 1 has a large deletion (1/2). One mild haemophilic with a missense mutation developed inhibitors and is a low responder. Four severe HA carried prothrombotic risk factors (4/35); 2 have FVL (1 is homozygous) and 2 have the PT G20210A. Conclusions. In our HA patients, the most frequent mutation type is mostly associated with gene rearrangements (IVS22 and IVS1) (19/32). Six out of 7 frameshifts identified are responsible for premature stop codons. The exception is the deletion c.3637delA associated with a mild phenotype, in which a reading frame correction probably occurs at the mRNA level. In the moderate and mild phenotypes the majority of mutations identified are missense transitional mutations. The mild haemophilic who developed inhibitors after treatment for surgery, has a mutation near an antigenic determinant region of FVIII. Amelioration of the phenotype is evident in the patient homozygous for FVL, as he only needs 2-3 treatments per year and his first hemorrhagic episode was traumatic, at the age of 5.

Table 1. New mutations identified at the Haemophilia Centre in CHC.

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<td>Intron 7 (acceptor)</td>
<td>ag/ATG&gt;cg/ATG</td>
<td>NS7</td>
<td>A1</td>
</tr>
<tr>
<td>Splicing</td>
<td>Primer-3</td>
<td>-</td>
<td>-</td>
<td>A1</td>
</tr>
<tr>
<td>Deletions</td>
<td>18 (inframer)</td>
<td>3 nt (5962-5964 del GAG)</td>
<td>A1</td>
<td>A3</td>
</tr>
<tr>
<td>Deletions</td>
<td>21-22</td>
<td>-</td>
<td>-</td>
<td>C1</td>
</tr>
<tr>
<td>Insertion</td>
<td>1</td>
<td>1T at codon 24</td>
<td>Frameshift</td>
<td>A1</td>
</tr>
<tr>
<td>Insertion</td>
<td>14</td>
<td>Duplic 13 bp (nt 836-840)</td>
<td>Frameshift</td>
<td>B</td>
</tr>
</tbody>
</table>

rt: nucleotide; delt: deletion; duplic.: duplication; bp: base pair; cd: codons.)

0061 MOLECULAR CLINICAL CORRELATION OF G6PD DEFICIENCY IN WESTERN SAUDI ARABIA

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Background. High frequencies of Glucose-6-Phosphate Dehydrogenase (G6PD) deficiency have been reported in most countries in the region. In Saudi Arabia, G6PD deficiency exists at variable frequency in different provinces of the country. G6PD deficiency may cause neonatal jaundice, sepsis, hemolytic anemia (farrin) following consumption of broad beans, and stress oxidative hemolysis occasionally can cause severe hemolytic anemia following treatment with specific drugs or participated by infection. Aims. The aim of this study was to investigate the mutation spectrum and clinical significance of the G6PD gene among population in western Saudi Arabia. Methods. A total of 492 unrelated native Saudi volunteers of both sexes (224 male, 268 female) were screened for G6PD deficiency by quantitative Methods. DNA was extracted from 42 G6PD-deficient Saudi subjects (36 males and 6 female). These subjects were screened for gene mutations using polymerase chain reaction/restriction fragment length polymorphism (PCR-RFLP). Screening included Mediterranean653C>T, and Aures143T>C, A202G A. Results. G6PD Mediterranean mutation 653C>T accounts for most cases of G6PD deficiency in Saudi nationals followed by G6PD Aures143T>C representing 38% and 17%, respectively. A new polymorphic variant (17%) has been identified during the course of this study although none of the samples showed A- mutation. Overall fre-
quency of G6PD deficiency is 0.265. G6PD Aureus showed more severe clinical manifestation. Summary/Conclusions. This study has characterized the molecular heterogeneity of G6PD variants among Saudis in the western Saudi Arabia suggesting significant gene flow. G6PD quantitative method and molecular characterization of G6PD deficiency shows high correlation with clinical manifestation.

0062

PREVALENCE OF NMP1 MUTATION IN AML AND MDS
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Background. A mutation on the nucleophosmin gene (NMP1) has been recently described on 25-35% of all AML patients. NMP1 is located on the 5q35 chromosomal region and this mutation, found on exon 12 of the NMP1 gene, causes the translocation of the NMP1 protein to the cytoplasm. This mutation is also linked to a wide spectrum of morphologic AML subtypes, normal karyotype, a better response to induction chemotherapy, and a higher prevalence of FLT3-ITD. Aims. To analyse the prevalence and prognostic value of the NMP1 mutation on AML and MDS patients. Methods. A total of 55 patients were studied and no promyelocytic AML were studied. These patients were previously examined for the FLT3-ITD mutation (5 M0, 8 M1, 10 M2, 12 M4, 4 M5, 1M6 and 15 not determined according to FAB criteria) and 1D M5. The average age of the patients ranged from 18 to 95 years. Screening for NMP1 mutation was undertaken by the Light Cycler system according to the Schnittger et al technique. Results. The NMP1 mutation was detected in 50.9% of patients with AML (15 out of 55), being this prevalence higher than that found for the FLT3-ITD, in the same population (23.8%). The 17 NMP1+ AML distributed as follows: 8 M1-M2 (44%), 4 M4-M5 (25%), and 5 not determined (33.3%). 68% of the NMP1+ patients had a normal karyotype, while 9% of them had cytogenetic anomalies. The FLT3-ITD mutation was found in 41.2% of the NMP1+ AML cases. The global mortality was analyzed with disregard to any risk factors, with a mortality of 67% in the NMP1+/FLT3-ITD group standing in clear contrast to a 100% death rate in the NMP1+/FLT3-ITD+ group. Within the limits of the group studied, no significant prevalence of the NMP1 mutation was observed regarding the sex of the patient. Conclusions. 1) The prevalence of the NMP1 mutation in our LMA group was 30.9%, and contrary to what has been described in literature, a higher incidence on M4 and M5 subtypes was not found. As recently published by Thiede et al, a higher incidence on M4 and M2 subtypes was found. 2) The NMP1 mutation prevalence was higher on MDS patients, which to our knowledge, had not been previously described in literature. 3) The other genetic anomaly most commonly associated to the NMP1 mutation was FLT3-ITD. 4) The screening for this mutation could be useful in the future when grouping patients with normal karyotype in a subgroup with better prognostic. 5) The high incidence of the NMP1 mutation in patients with MDS could suggest a role for this gene in the pathogenesis of this disease.

0063

THE COMPARISON OF THE RESULTS OF MOLECULAR MONITORING OF IMATINIB THERAPY IN CML BCR-ABL POSITIVE PATIENTS USING qPCR AND TWO CONTROL REFERENCE GENES
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University of Medical Sciences, POZNAN, Poland

Background. The tyrosine kinase inhibitor imatinib mesylate (Gleevec, STI571) has proven to be an effective new therapy for patients with chronic myeloid leukemia (CML). Quantitative reverse transcription-polymerase chain reaction (qPCR) for detection of BCR-ABL transcripts is frequently used for monitoring patients. In available publications there is still no homogenous and precision recommendations for standardization of the detection of BCR-ABL transcripts. The main doubts concern choice of qPCR machine and control gene. The ABL gene is definitely recommended, but other genes (like BCR, GUSnpr-β-glucuronidase, β-2-microglobulin, G6PD etc) are also acceptable. Aims. In this study we compared the efficiency of the qPCR methods using two control reference genes (GAPDH and CAB1) for monitoring of effectiveness of imatinib therapy. Methods. The study group consisted of 33 patients (16 female, 17 male) with confirmed diagnosis of CML (32 in chronic phase and 1 in accelerated phase). 10ml peripheral blood was taken every 3 months. Total cellular RNA was obtained by phenol-chloroform extraction, isopropanol precipitation and washing with 700 m/mL ethanol. Samples were stored in temperature up do -80°C. The level of BCR-ABL transcripts was measured in Rotor Gene 2000 machine (Corbett Research). The amounts of BCR-ABL were also calculated from a 5-bef ready-to-use reference dna strip containing 8 defined amounts of BCR-ABL as a quantification control in a range of 100,000 copies/run (RoboGene M-bcr cdNA Quantification Module, Roboscreen). Values were normalized for expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) using the RoboGene GAPDH cdNA Quantification Module and c-ABL using the RoboGene c-ABL cdNA Quantification Module. Statistical analysis: Spearman’s rank test was used to assess correlations between qPCR values using different control genes. Results. Median values of qPCR and the availability of results at baseline, 3, 6 and 9 months are shown in Table 1.

| Table 1 |
|-----------------|----------------|----------------|----------------|
|                  | Baseline | 3 months | 6 months | 9 months |
| qPCR (BCR-ABL/GAPDH) median range | 0.6 | 2.5 | 0.52 | 0.21 |
|                   | 0.006-0.8 | 0.004-2.24 | 0.004-2.8 | 0.0005-0.7 |
| qPCR (BCR-ABL/cABL) median range | 2.53 | 0.15 | 0.53 | 0.53 |
|                   | 0.02-14.23 | 0.003-8.1 | 0.006-7.44 | 0.001-3.25 |

There was a highly significant correlation between BCR-ABL/GAPDH and BCR-ABL/cABL values (r=0.781, p<0.001, Spearman’s rank test). In addition we also noticed a good correlations between number of BCR-ABL copies and BCR-ABL/cABL ratio (r=0.38, p<0.001) and between number of BCR-ABL copies and BCR-ABL/cABL ratios (r=0.49, p<0.001) as well. Conclusions. Our data suggest that measurement of BCR-ABL/GAPDH and BCR-ABL/cABL ratios are the equivalent and useful methods of molecular monitoring of CML treatment. BCR-ABL/GAPDH ratio significantly correlate with the value of BCR-ABL/cABL ratio. However, in spite of generally acceptable recommendations, we found also good correlations between the absolute number of BCR-ABL copies/run and BCR-ABL/GAPDH or cABL ratios.

0064

IMPROVED EFFICIENCY IN MOLECULAR DIAGNOSIS OF T(14;18)(Q32;Q21) AND T(11;14)(Q13;Q32) USING MULTIPLE- AND LONG DISTANCE INVERSE-PCR
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1Instituto Nacional de Saúde, LISBOA, Portugal,2Lab. de Hematologia, Hosp. Sta Cruz, CARNAXIDE, Portugal

Translocations t(14;18)(q32;q21) and t(11;14)(q13;q32) are cytogenetic hallmarks of follicular (FL) and mantle cell lymphoma (MCL), respectively. These translocations target the IgH locus, suggesting a rearrangements of IGH alleles and a BCL2 heavy chain (IGH) locus at 14q32 resulting in juxtaposition of the BCL2 gene (18q21), or the BCL1 locus (11q13), to IGH. However, BCL2 and BCL1 breakpoints are only partially clustered so that a significant proportion of BCL2-IGH and BCL1-IGH fusion genes are not detected using standard PCR techniques. In order to identify and characterize the highest number of breakpoints, we used two sequential molecular approaches. First, a multiplex-PCR (M-PCR) assay was designed that allowed a single-tube amplification of the most common breakpoints in BCL2 (MBr and mcr regions) and BCL1 (MTC region), in addition to a control BCL2 fragment. Using this assay on 20 FL and 11 MCL diagnostic samples without knowledge of cytogenetic status, we observed 14 FL patients (70%) with BCL2-IGH (11 MBr and 3 mcr breakpoints) and 6 MCL patients (55%) with BCL1-IGH fusion genes. Sequencing of the corresponding PCR products confirmed the predicted fusion gene in every sample, revealing that M-PCR unequivocally identified the fusion gene in each patient. As a second approach in the remaining 11 negative patients, long distance inverse-PCR (LDI-PCR) was used to identify BCL2-IGH and BCL1-IGH rearrangements with variant breakpoint locations. PCR products corresponding to rearranged IGH alleles were cloned, sequenced, and the respective sequences compared to the GenBank database. We found a BCL2-IGH fusion in 5 patients with FL and a BCL1-IGH fusion in 3 patients with MCL (Table 1). The sequence of each fusion gene was confirmed by conventional PCR using a JH primer in combination with a BCL1 or BCL2 breakpoint region-specific primer. In BCL2, one of the breakpoints localized 1.5 kb upstream of the mcr region while the remaining localized at the intermediate cluster region and differed by 2 bp only. One of these breakpoints was identical to the one previously reported by others in 2 rare patients, suggesting that this particular sequence constitutes a micro cluster region of breakpoints.
trast, BCL1 breakpoints were scattered within a 15 kb sequence at a distance of approximately 90 kb downstream of the MTC region. Two breakpoints localized within the promoter region of cyclin D1 at a distance of 1360 and 2870 bp from the transcription start site. In IGH, one translocation involved a D2-2/JH6 rearrangement, while the others showed involvement of either JH4 (4 cases) or JH6 (1 case). In summary, LDI-PCR was instrumental in the identification of an additional 3 breakpoints in BCL1 (80%) and 2 in BCL2 (50%) in patients who showed no breakpoints at the main cluster regions. Overall, BCL2-IGH fusion was detected in 17 patients with FL (85%) and BCL1-IGH fusion was found in 9 patients with MCL (82%). We conclude that M-PCR was found in 9 patients with FL (85%) and BCL1-IGH fusion breakpoints localized within the promoter region of cyclin D1 at a distance of approximately 90 kb downstream of the MTC region. Two

### Table 1. BCL1- and BCL2-IGH sequences identified by LDI-PCR.

<table>
<thead>
<tr>
<th>Acc. number</th>
<th>Gene</th>
<th>Gene sequence</th>
<th>N region</th>
<th>IGH sequence</th>
<th>JH</th>
</tr>
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<tbody>
<tr>
<td>DQ401134</td>
<td>BCL1</td>
<td>GAGACTGGAAAACCTTTC</td>
<td>D2-2/JH6</td>
<td>GTTGACTACTGGGGCCAG</td>
<td>JH4</td>
</tr>
<tr>
<td>DQ403540</td>
<td>BCL1</td>
<td>TGGCATACAAACGCG</td>
<td>cccctaat</td>
<td>ACTGGGGCCAGGGAACC</td>
<td>JH6</td>
</tr>
<tr>
<td>DQ421758</td>
<td>BCL1</td>
<td>CTGGGCGGCGACTGG</td>
<td>ggcttc</td>
<td>GAGACTGGGCGGCGAGG</td>
<td>JH4</td>
</tr>
<tr>
<td>DQ400339</td>
<td>BCL2</td>
<td>GTGAGGTGGGACATC</td>
<td>ggcttc</td>
<td>GAGACTGGGCGGCGAGG</td>
<td>JH4</td>
</tr>
<tr>
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<td>ggcttc</td>
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</table>

**Molecular targeting and gene therapy**

0065

THE EFFECT OF HISTONE DEACETYLASE INHIBITORS ON B-CELL DIFFERENTIATION IS NOT UNIQUE FOR TEL-AML1 POSITIVE ACUTE LYMPHOBlastic LEUKEMIAS

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The TEL-AML1 fusion is the most frequently found translocation in childhood pediatric acute lymphoblastic leukemia (ALL). TEL-AML1-positivity is associated with L-asparaginase sensitivity and favorable clinical outcome in pediatric ALL. The fusion protein is thought to recruit co-repressors and histone deacetylases (HDACs), which in turn lead to transcriptional repression of AML1-responsive genes. FK228 (depsipeptide) is an HDAC inhibitor that affects the chromatin configuration and, as a consequence, affects gene transcription. We investigated whether HDAC inhibitors may be used to target TEL-AML1 positive ALL in children. To this aim, the in vitro cytotoxic effect of FK228 as single agent and in combination with L-asparaginase was tested in leukemic cells obtained from TEL-AML1 positive and negative children with ALL at initial diagnosis (by MTT-assay). In addition, the effect of FK228 on B-cell differentiation was analyzed by monitoring changes in differentiation marker expression using flow cytometry. Our data indicate that leukemic cells of 14 TEL-AML1 positive and 15 negative B-lineage ALL cases were both more in vitro sensitive to FK228 than normal bone marrow cells (p=0.05). FK228 exposure induced the differentiation of leukemic cells into more mature precursor B-cells. However, the in vitro cytotoxicity of FK228 did not differ between both ALL subtypes. FK228 had an additive but not a synergistic effect on in vitro sensitivity to L-asparaginase in both ALL subtypes. In conclusion, FK228 induces differentiation in children with B-lineage ALL, but its effect is not selective for TEL-AML1 rearranged B-lineage ALL only.

0066

IMATINIB MESYLATE CAN INDUCE MOLECULAR COMPLETE REMISSION IN IDIOPATHIC HYPEREOSINOPHILIC SYNDROME. A PHASE II MULTICENTRIC ITALIAN CLINICAL TRIAL

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Idiopathic hyper-eosinophilic syndrome (HES) is a rare hematological disorder characterized by persistent peripheral blood greater than 1,500 cells/mL lasting for more than 6 months, in the absence of other apparent aetiologies for eosinophilia with signs and symptoms of organ involvement. HES may be a reactive condition or a chronic myeloproliferative disorder with evidence of clonal proliferation, in which latter case it is usually referred to as chronic eosinophilic leukemia (CEL). Patients with HES generally have a poor prognosis, but the course of the disease may be variable. Severe visceral complications, including cardiopathies, are common and are often fatal illness. Treatment of HES includes corticosteroids, chemotherapeutic agents and, more recently, interferon-α (IFN-α). Cools et al. reported the involvement of PDGFRα, fused with FIP1L1, in a number of HES patients responsive to imatinib therapy. We treated with imatinib mesylate (100 to 400 mg daily) 39 patients affected by Hyper-Eosinophilic Syndrome (HES) enrolled in a multicentric Italian phase 2 clinical trial. All the patients were studied by molecular analysis for expression of FIP1L1-PDGFRα, TEL-PDGFRα, FGF1-BCR and BCR-ABL chimerical transcripts. 23 patients (59%) were positive for the FIP1L1-PDGFRα rearrangement. Rapid, hematological complete responses (HCR) were recorded after one month of therapy in all FIP1L1-PDGFRα positive. In 36 patients resulted negative for FIP1L1-PDGFRα rearrangement we observed 8 (22%) hematological improvement (HI) and one HCR (HI-HCR 25%). Furthermore, a molecular complete remission (defined as the disappearance of FIP1L1-PDGFRα at qualitative RT-PCR evaluation) was recorded in all but one patients of the 23 valuable after three months of therapy, and we
obtained molecular remission in 18 out of 21 evaluable patients after six months of therapy. After one year of imatinib therapy, all 12 evaluable patients showed disappearance of the rearrangement. No significant toxicity was seen during the treatment. The median follow up was 7 months (range: 2-41). This is the largest series of HES patients treated with Imatinib with strong evidence of hematological and molecular effectiveness and absence of significant toxicity. This phase II study supports the use of Imatinib as first line therapy in IPF111-PDGFRA rearrangements positive HES patients. Acknowledgments. COFIN 2003 (Molecular therapy of Ph+ leukemias), by FIRB 2001, by the University of Bologna (60%), by the Italian Association for Cancer Research (A.I.R.C.), by the Italian National Research Council (C.N.R), by Fondazione Del Monte of Bologna and Ravenna (Italy) and A.I.L. grants, LeukemiaNet grants.

0067
SIRNA VERSUS LOCKED NUCLEIC ACID RNA ANTAGONISTS: STAY SINGLE!

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By the incorporation of Locked Nucleic Acid (LNA), a conformational analogue of RNA, single stranded LNA/DNA oligonucleotides can be created which mimic RNA and have unrivaled gene silencing ability. Much discussion has centred on the utility and benefits of siRNA in both target validation and as a therapeutic option. This has been driven by significant publications including that of Soutcheck et al. (Nature 432, 173-177 2004), which demonstrated liver targeting as well as a siRNA against ApoB was tethered to a cholesterol moiety. We therefore sought to compare single-stranded oligonucleotide antagonists containing LNA with siRNA against this target in both in vivo/in vitro settings. The same motif used in the Soutcheck study was targeted with the LNA molecule, and the activity of unmodified siRNA was compared to the cholesterol-linked and native LNA molecules in their ability to down-regulate ApoB expression. LNA (SPC3197) inhibited ApoB expression by 90% while an equimolar concentration of siRNA was ineffective in the liver and jejunum. Cholesterol linked siRNA was partially effective in the jejunum (50% reduction in mRNA). Only the LNA mediated inhibition of ApoB expression was paralleled by decreases in serum cholesterol in the host animal. In a second model, siRNA molecules targeting Hif-1α mRNA (Yu et al. J. Lab Invest 84, 553-561 2004) were compared to a single-stranded LNA/DNA mixmer targeting Hif-1α, SPC2968. In in vitro analyses of these 2 molecules were equally potent. However, in a murine model the increased half-life of the LNA molecules translated to a potent inhibition of Hif-1α as measured by QPCR. This effect was observed in jejunum and liver, and persisted for at least 4 days. Hif-1α inhibition mediated by siRNA was not seen in any tissue analysed. Overall we demonstrate clear superiority of single chain LNA based RNA antagonists over siRNA for the therapeutic molecules.

0068
DUAL SRC/ABL INHIBITOR SKI-606 BINDING MODE IN BCR-ABL KINASE HYPOTHEZIZED ON THE BASIS OF MOLECULAR DOCKING STUDIES

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Background. SKI-606 is a novel 4-anilino-3-quinoxincarbonitrile Src and Abl kinase inhibitor. SKI-606 has been shown to be a potent antiproliferative and proapoptotic agent when tested on Bcr-Abl-positive cell lines. The remarkable efficacy of SKI-606 against chronic myeloid leukemia (CML) cells in culture was mirrored by its activity in vivo against CML xenografts: K562 tumors regressed in nude mice when SKI-606 was administered per os once daily over a 5-day period. The crystal structure of the Bcr-Abl kinase complex with SKI-606 has not yet been determined and the mode of binding of this inhibitor is therefore unknown. Moreover, there are currently no published data on the ability of SKI-606 to bind and efficiently inhibit the Bcr-Abl mutants known to confer resistance to imatinib. Aims. In this study, we used a molecular docking approach to a) determine SKI-606 binding mode to the wild-type (wt) form of the Bcr-Abl kinase; b) hypothesize SKI-606 binding mode to the more frequent, clinically relevant Bcr-Abl mutants known not to be inhibited by imatinib; c) predict which novel mutant forms might emerge and interfere with SKI-606 binding. Methods. Modelling of the human Abl kinase was performed with the program Modeller v7.7 (http://salilab.org/modeller) adopting the highly related Mus musculus Abl homologue as a template structure (PDB: 1OPJ, 0.175nm resolution). Chemscketch (http://www.acdlabs.com) was used to build a three-dimensional model of SKI-606. Flexible docking of the ligand to the protein was performed with Autodock v3.0 (http://www.scripps.uchicago.edu). Results. SKI-606 binds the active site of Bcr-Abl with the activation loop in the active (open) and inactive (closed) conformation (the latter is the one to which imatinib binds). According to our results, the interaction between SKI-606 and Bcr-Abl seems to be more stable when the activation loop is in the inactive conformation. The consequent structural study of SKI-606 modeled into wt-Bcr-Abl ATP binding site highlighted the variable location of the binding site over a spherical environment of 0.5nm centered on SKI-606: Y253, T315 and F359 (residues numbered according to ABL exon I splice variant). The binding of SKI-606 to the eight Bcr-Abl mutants which are most frequently implicated in clinical resistance to imatinib mesylate was also studied: G250E, Y253H, E255K, T315I, M351T, F359V, H396R. Our results indicated that SKI-606 retains the ability of efficiently binding all the above mentioned Bcr-Abl variants with the exception of the T315I mutant. Finally, we identified six potential residues around SKI-606 that, if mutated, could potentially be able to interfere with the SKI-606/Bcr-Abl interaction: a) the charged residues K271, D381 and H361; b) the hydrophobic/sialphatic residues V299, A380 and M319. Conclusions. Pre-clinical data suggest that SKI-606 is a promising second-generation kinase inhibitor with potent antiproliferative and proapoptotic effects on CML cells. Our docking experiments indicate that SKI-606 may prove effective in imatinib-resistant patients since it is expected to retain the ability to bind several known to confer resistance to imatinib.

0069
EFFECTIVE INHIBITION OF BCR/ABL KINASE WITH TETRAMERIZATION DOMAIN DERIVED PEPTIDES MAPS TO COILED-COIL HELIX-ALPHA-2

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Background. As a result of the (9;22), more than 95% of CMLs and 20-25% of adult ALLs express the p210(BCR-ABL) or the p185(BCR-ABL) fusion protein respectively. The BCR portion of the fusion protein harbors an N-terminal coiled-coil (CC) domain which induces tetramerization of BCR. The CC contains two helical motifs - Helix-α-1 and Helix-α-2 - and assembles to dimers with antiparallel orientation which associate to form tetramers. Helix-α-2 contributes the majority of the dimer and tetramer interface. The BCR mediated tetramerization of ABL in the fusion protein leads to the constitutive activation of the ABL kinase. The subsequent permanent activation of multiple downstream signaling pathways induces the leukemic phenotype. Targeting specific regions of the BCR/ABL fusion protein was designed to block the formation of the fusion protein and to inhibit the BCR/ABL kinase activity. Here we studied the inhibitory effects of the CC subdomain Helix-α-2 which harbors the majority of the protein-protein interface. We aimed to i) reduce the molecular weight of the inhibitory peptides and ii) thereby to map the inhibitory effects of CC-derived peptides to CC-substructures. Methods. Helix-2-GFP fusion peptides were coexpressed with BCR/ABL in the IL-3 dependent cell line Ba/F3 using a bicistronic retroviral vector. The interaction of the Helix-2 and BCR/ABL was checked by pull-down assays. The IL-3 independent proliferation of BCR/ABL expressing Ba/F3 cells in presence of Helix-2 was studied in presence and absence of Gleevec. Anti-phospho-ABL specific immunoblotting was used to reveal the BCR/ABL autophosphorylation in these cells. All studies were performed with the previously published CC-GFP fusion
peptide as control. Results. Here we report that i) Helix-α-2 interacts with BCR/ABL to the same extent as the complete CC domain; ii) Helix-2 like CC decreases the autophosphorylation of BCR/ABL in transduced Ba/F3 cells; iii) Helix-2 increases the sensitivity of IL-3 independent BCR/ABL expressing Ba/F3 cells towards Gleevec to the same extend as CC; iv) Helix-2 shows no inhibitory effects on IL-3 independent Ba/F3 cells expressing activated c-Kit. Conclusion. Taken together these results show that Helix-α-2 specifically targets the tetramerization-interface of BCR/ABL. The peptides inhibit the ABL-kinase activity and enhance the inhibitory effects of Gleevec. This study provides important information for the use of Helix-α-2 as lead structure in the rational design of small molecule inhibitors of BCR/ABL tetramerization.

0070

**SINGLE-AGENT SU11657, A NOVEL FLT3 INHIBITOR, SHOWS BIOLOGIC ACTIVITY IN ACUTE MYELOID LEUKEMIA CELLS IN VITRO**

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Background. Fms-related tyrosine kinase3 (FLT3) is one of the most commonly mutated gene in human acute myeloid leukemia (AML) and has implicated in its pathogenesis. Constitutive activation of the FLT3 receptor tyrosine kinase, has been linked either by internal tandem duplication (ITD) of the juxtamembrane region or by point mutation in the second tyrosine kinase domain (TKD). Aims. The purpose of the study was to evaluate, in vitro, the effect and the biological activity of SU11657 (Pfizer), a new compound FLT3 kinase inhibitor. SU11657 was investigated on human cell lines from AML patients (MV4-11 and HL-60) and blast specimens of patients, using a wide range of concentrations (1nM-10 μM). Methods. FLT3 expression levels were evaluated by flow cytometry. Furthermore, to evaluate the effect of SU11657 we analyzed the cytotoxicity, induction of apoptosis and inhibition of cell proliferation by flow cytometry. The antiproliferative and cytostatic effects of SU11657 were confirmed by analysis of signal transduction. HL-60 cell line served as a control as it expresses a wild type receptor. MV4-11 is a cell line that expresses a naturally internal tandem duplication (ITD) in homozygous form. Results. In HL-60 does not show relevant effect after treatment with SU11657. Instead, in MV4-11 we observed a decrease dose-dependent in cell viability after treatment with SU11657. The effects of this compound on cell cycle progression show an accumulation of G1/S phase and an induction of apoptosis at 1-10nM concentration after 24h of treatment. First we observed a dephosphorylation of FLT3 on Tyr(959) in whole cells extract from MV4-11 cells after treatment with SU11657 100nM. We also demonstrated a hypophosphorylation of AKT on Ser(473) and a consequently dephosphorylation of BAK on Ser(136) at nanomolar concentration. We observed a dephosphorylation of STAT-5 to 100nM of SU11657 at 24h. We evaluated the effects of this new compound in AML primary progenitors that showed FLT3-ITD, FLT3-TKD and FLT3-vit. In the patients with mutation ITD and TKD was evident a modification of cell cycle progression with a decrease in G2/M phase and an increase of subdiploid peak. The effect of SU11657 in patients FLT3-wt was not relevant. Conclusions. Due to its FLT3 inhibitory activity, SU11657 represent promising compound for clinical studies in FLT3 mutation AML. Study of signal transductions and gene profile expression will contribute to further understanding of the drug mechanisms. Acknowledgments. COFIN 2005 (Myelodysplastic syndromes: pathogenetic models and promise of new therapies), COFIN 2006 (Targeting of leukemia), by I.R.C.C.S. I.R.C.C.S. (GRF 2001, by the University of Bologna (60%)), by the Italian Association for cancer research (A.I.R.C.), by the Italian National Research Council (C.N.R.), by Fondazione Del Monte di Bologna e Ravenna (Italy) and A.I.L. grants.

0071

**POTENTIAL THERAPEUTIC APPROACH OF RECOMBINANT TRAIL IN CML IN BLAST CRISIS**


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Tumour necrosis factor-related apoptosis-inducing ligand (TRAIL/APO2L) has been shown to induce apoptosis in a number of tumour cell lines as well as in some primary tumours whereas cells from most normal tissues are highly resistant to TRAIL-induced apoptosis. TRAIL is a member of the TNF super family and exerts its activity by inducing one of two death systems: the death receptor (DR4/TRAIL-R1 and DR5/TRAIL-R2) and three decoy receptors (DR3/TRAIL-R3, DRc2/TRAIL-R4 and osteoprotegerin/OPG). Although these receptors are characterized by a high sequence homology in their extracellular domains, only DR4/TRAIL-R1 and DR5/TRAIL-R2 contain a functionally active cytoplasmic death domain that allows an apoptotic response upon TRAIL stimulation. The biological significance of TRAIL as mediator of innate and specific immunity against transformed and virus-infected cells has been clearly documented by several reports. As membrane-bound TRAIL seems to have tumour-selective pro-apoptotic activity, the potential use of recombinant soluble forms of this molecule as cancer therapeutic is presently being exploited in several pre-clinical and preliminary clinical trials. In particular, a mouse apoptosis-inducing ligand (TRAIL/APO2L) has been very successful in the treatment of chronic myelogenous leukaemia (CML). However, the majority of patients achieving cytogenetic remissions with imatinib treatment have molecular evidence of persistent disease, and residual BCR/ABL+ progenitors can be detected. There is a need to develop new approaches that enhance elimination of malignant progenitors in imatinib-treated patients. The aim of this work is to study the susceptibility of TRAIL-induced apoptosis in hematological neoplasias, namely in Chronic Myeloid Leukaemia (CML) in blast crisis. For this purpose K562 cells were incubated in absence and presence of different concentrations of recombinant TRAIL, alone or plus imatinib or MG262 (a proteasome inhibitor) during 72 hours. Cell death was evaluated by Annexin V/propidium iodide incorporation and detected by flow cytometry. The expression of TRAIL receptors and the proteins involved in apoptosis regulation, namely Bax, Bcl-2, p53 and survivin was analysed by flow cytometry using monoclonal antibodies. Preliminary results show that TRAIL, as single agent does not decrease significantly K562 cell viability. However when the cells are previously treat with Imatinib or MG262 in lower concentration than IC50, we observe a potentiation of the cytotoxic effect. The increase in this cytotoxicity seems to occur by activation of apoptotic pathways as we have observed morphological characteristics of apoptosis and an increase in annexin V positive cells. The mechanisms involved may be related with the observed increase in Bax and/or in DR4 receptor expression. These results support the idea that imatinib and proteasome inhibitors may potentiate the apoptosis induced by TRAIL. On the other hand, the increase in imatinib efficacy, may allows its clinical use in lower doses with lesser toxicity. Acknowledgments. Prof. Catarina Oliveira, Santos Rosa and Lina Carvalho Directors of Biochemistry, Immunology and Anatomopathology Institutes, respectively, Faculty of Medicine, University of Coimbra. This work is supported by GAI and CIMAGO.

0072

**RCE1 DEFICIENCY ACCELERATES THE DEVELOPMENT OF A K-RAS-INDUCED MYELOPROLIFERATIVE DISEASE**

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Background. Ras proteins undergo three processing steps that each enhance membrane affinity: farnesylation, endoproteolysis, and carboxyl methylolation of a carboxyl-terminal CAAX motif. Ras converting enzyme 1 (Rce1) catalyzes the second step the endoproteolytic removal of the three amino acids (AAX) downstream of the farnesylated cysteine. We have explored Rce1 as a potential drug target in the treatment of Ras-induced malignancies. We previously showed that inactivation of the Rce1 gene blocks the plasma membrane targeting of Ras and inhibits Ras transformation of fibroblasts and skin carcinoma cells. In vivo, transformation of fibroblasts and skin carcinoma cells induced malignancies. We previously showed that inactivation of the Rce1 gene blocks the plasma membrane targeting of Ras and inhibits Ras transformation of fibroblasts and skin carcinoma cells. In vivo, transformation of fibroblasts and skin carcinoma cells induced malignancies. We previously showed that the absence of Rce1 rendered Ras-transformed cells hypersensitive to a farnesyltransferase inhibitor. Currently, nothing is known about the impact of inhibiting Rce1 on the development of K-Ras-induced malignancies in vivo. Aims. Our aim was to test the hypothesis that inactivation of the Rce1 gene concentrations of recombinant for an oncogenic mutation, and lethality of a K-Ras-induced myeloproliferative disease (MPD) in vivo. To accomplish this, we used Cre-loxP1 techniques in mice to simultaneously activate the expression of oncogenic K-Ras to induce MPD and inactivate the expression of Rce1. In this way, we could determine if the absence of Rce1 would block the development of K-Ras-induced MPD. Methods. We use mice that are heterozygous for an oncogenic mutation, and lethality of a K-Ras-induced myeloproliferative disease (MPD) in vivo. To accomplish this, we used Cre-loxP1 techniques in mice to simultaneously activate the expression of oncogenic K-Ras to induce MPD and inactivate the expression of Rce1. In this way, we could determine if the absence of Rce1 would block the development of K-Ras-induced MPD.
Optimizing T Cell Receptor Gene Transfer to Virus-Specific T Cells for Clinical Application

M. Griffioen, H.M. van Egmond, M.A.W.G. van der Hoon, R.S. Hagedoorn, M. Kester, R. Willemze, J.H.F. Falkenburg, M.H.M. Heemskerk
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Patients with relapsed or resistant hematological malignancies after allogeneic stem cell transplantation (alloSCT) can be successfully treated by donor lymphocyte infusions (DLI). However, Graft-versus-Host Disease (GVHD) remains an important cause of morbidity and mortality. We have previously shown that functional T cells with redirected anti-leukemic effects, like GVHD, occur. Since tetrameric complexes are currently not available, we explored the feasibility of using synthetic peptide pools as a selection marker/suicide gene.

### OPTIMIZING T CELL RECEPTOR GENE TRANSFER TO VIRUS-SPECIFIC T CELLS FOR CLINICAL APPLICATION

**Results.**

1. We find inhibition of Rce1 in vivo actually accelerates the development of K-Ras-induced MPD.
2. The inactivation of Rce1 further resulted in a massive release of immature myeloid cells from the bone marrow which likely contributed to the early demise of the mice.
3. Conclusions. Our hypothesis was that inhibition of Rce1 may be an effective strategy to block the development of K-Ras-induced malignancies. This hypothesis, which was based on comprehensive in vitro studies, was dashed by the current experiments. We find that inhibition of Rce1 in vivo actually accelerates the development of K-Ras-induced MPD. Future studies will evaluate the mechanism behind this totally unexpected result.

### 0073

**OPTIMIZING T CELL RECEPTOR GENE TRANSFER TO VIRUS-SPECIFIC T CELLS FOR CLINICAL APPLICATION**

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Patients with relapsed or resistant hematological malignancies after allogeneic stem cell transplantation (alloSCT) can be successfully treated by donor lymphocyte infusions (DLI). However, Graft-versus-Host Disease (GVHD) remains an important cause of morbidity and mortality. We previously showed that functional T cells with redirected anti-leukemic reactivity can be generated by transfer of T cell receptors (TCRs) specific for minor histocompatibility antigens (mHags) to virus-specific T cells. Adoptive transfer of virus-specific T cells to patients treated with alloSCT has a minimal risk for GVHD. The aim of this study is to develop a method for the generation of TCR-transduced virus-specific T cells for cellular immunotherapy of patients with relapsed hematological malignancies after alloSCT. Various single retroviral vectors were constructed containing the α and β chains of the HA-2 TCR linked by an IRES or 2A-like sequence. Introduction of a 2A-like sequence allows additional linkage of the human low affinity nerve growth factor receptor (NGFR) or CD20 selection marker genes by an IRES element. Inclusion of a selection marker gene allows purification of TCR-transduced cells, thereby reducing the risk for GVHD. The human CD20 gene also functions as suicide gene, allowing elimination of transduced cells in vivo when undesired side effects, like GVHD, occur. Since tetrameric complexes are currently not GMP-grade available, we explored the feasibility of using synthetic peptide pools for the generation of TCR-transduced virus-specific T cell lines. From various human individuals, CD8+ cells were isolated and stimulated with a mixture of CMV and EBV peptides. At day 8, CD8+ cells were transduced with retroviral vectors encoding the HA-2 TCR. Due to selective expansion upon peptide stimulation, all cell lines were shown to contain high cumulative percentages of virus-specific T cells (20-80%) as well as TCR-transduced cells (10-50%) at day 8. Moreover, significant numbers of specific T cells were obtained, demonstrating that this strategy is feasible for adoptive cellular immunotherapy. Highest levels of TCR expression and HA-2-specific lysis were obtained with retroviral vectors containing two genes encoding the TCR α and β chains linked by an IRES or 2A-like sequence. Upon linkage of a third gene, TCR expression and HA-2-specific lysis were slightly (NGFR) or significantly (CD20) reduced. Since highly-enriched virus-specific T cells will be used for adoptive transfer, GVHD is not likely to develop and inclusion of a selection marker/suicide gene not strictly required. Therefore, for clinical applications TCR-transduced virus-specific T cells, a retroviral vector containing two genes encoding the TCR α and β chains in the absence of a selection marker/suicide gene is preferable.

### 0074

**DUAL SPECIFIC T CELLS CHANGE THEIR T CELL RECEPTOR (TCR) CELL SURFACE DISTRIBUTION UPON TCR TRIGGERING**

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Tumor transfer to engineer tumor specific T cells may be an alternative strategy for adoptive immunotherapy. When virus specific T cells are used for TCR transfer, we hypothesize that due to the latent presence of viral antigens the survival of TCR-transferred dual specific T cells will improve. However, repetitive stimulation of the endogenous TCR may lead to selection of dual specific T cells with high expression of the endogenous TCR and low expression of the introduced TCR. To address this issue, we used CMV-specific T cells that were transduced with the hematopoietic minor histocompatibility antigen HA-2 specific TCR. The dual specific T cells were repetitively stimulated either via their endogenous virus specific TCR or via the introduced HA-2 specific TCR and analysed by FACS after each stimulation. In time, the expression of the endogenous and introduced TCRs measured with CMV and HA-2 tetrameric complexes diverged. Repetitive stimulation of the endogenous TCR skewed the dual specific T cells towards a cell population that primarily expressed the endogenous TCR. In contrast, repetitive stimulation of the introduced TCR skewed the T cells towards T cells that primarily expressed the introduced TCR. However, this divergence in tetramer stainings appeared to revert quickly after stimulation via the other TCR, suggesting that this divergence was the result of a difference in TCR surface distribution and not of selective outgrowth of different T cells. To rule out that differences in tetramer stainings were the result of selective outgrowth, T cells were sorted after repetitive stimulation expressing primarily the endogenous or introduced TCR. These cells were subsequently stimulated on the endogenous or introduced TCR and analysed for TCR expression and functional activity. Results indicate that no selective outgrowth occurred, but that T cells change their TCR cell surface distribution dependent on which TCR is triggered. In conclusion, virus specific TCR-transferred T cells repetitively stimulated via their endogenous TCR phenotypically seemed to express primarily the virus specific TCR. However, when restimulated on the introduced TCR, T cells reverted into cells with high expression of the introduced TCR that exerted potent HA-2 specific anti-leukemic activity, indicating that these dual specific T cells are useful for clinical applications.

### 0075

**RELEVANCE OF MEK/ERK INHIBITION IN THE MAINTENANCE OF THE PML/RAR-ALPHA INTEGRITY IN ACUTE PROMYELOCYTIC LEUKEMIA**

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The mitogen-activated protein kinase MEK/ERK and phosphatidylinositol 3-kinase PISK/Akt pathways are involved in proliferation, inhibition of apoptosis, differentiation and cell survival. These pathways are frequently activated in Acute Promyelocytic Leukemia (APL), and play a key role in the survival of neoplastic cells. The hallmark of APL is the t(15;17), which leads to the expression of the PML/RARα fusion protein. PML/RARα is the central leukemia-inducing lesion in APL and is directly targeted by all-trans retinoic acid (ATRA) as well as by arsenic trioxide (ATO), both compounds able to induce clinical complete remissions. In addition, the precise intracellular mechanisms of action of ATRA and ATO remain unclear. The purpose of this study was to evaluate: 1) the effects induced by the downmodulation of MEK/ERK and PISK/Akt pathways on the PML/RARα expression, and 2) the role of these pathways in the PML/RARα degradation induced by ATRA and low-dose ATO in APL cells. PML/RARα expression was analyzed by western blot after treatment of the promyelocytic cell line NB4 with the selective pharmacological inhibitors of MEK/ERK and PISK/Akt pathways, PD98059 (20 μM) and LY294002 (20 μM), respectively, given either alone or in combination with ATRA (1uM) or ATO (0.1 μM). The inhibitor of the MEK/ERK pathway caused a significant degradation of PML/RARα, which was reversed after treatment with the caspase inhibitor z-VAD-fmk (50 μM), indicating that PML/RARα degradation induced by downmodulation of MEK/ERK seems to be a mechanism dependent on caspase activation. In addition, the combined treatment with ATRA or
ATO and PD003059 further reinforced the PML/RARA degradation induced by ATRA or low doses of ATO of the other hand, the con- binined treatment with arsenic trioxide and LY294002 reversed oncoprotein degradation induced by ATO alone, thus suggesting that the PI3K/Akt pathway might mediate the degradation of PML/RARA induced by low-dose ATO. Taken together our findings suggest that MEK/ERK activation might be responsible for the maintenance of the PML/RARA protein levels. Further studies are warranted to more clearly address the effects of siRNA-mediated MLL-AF4 suppression on the expression and function of this chromosomal translocation in t(4;11)-positive leukaemic cell lines. The reciprocal chromosomal translocation t(4;11) (q21;q25) results in the expression of two fusion-proteins, MLL-AF4 and AF4-MLL, and marks a therapy-resistant infant acute lymphoblastic leukemia (ALL) sub-type. Here, we addressed the effects of siRNA-mediated MLL-AF4 suppression on the expression of putative target genes in t(4;11)-positive leukemic cell lines. Methods. t(4;11)-positive cell lines were electrophorized with MLL-AF4, HOXA7 or control siRNAs. Gene expression was analyzed by real time RT PCR, intracellular protein-DNA interactions by chromatin immuno- precipitation, and promoter methylation status by bisulfite sequencing. Results. SiRNA-mediated suppression of both MLL-AF4 and of HOXA7, a putative target gene of MLL-AF4, diminishes hTERT transcript levels. In particular, the knock-down of MLL-AF4 is associated with decreases in the methylation status of the hTERT promoter. Furthermore, chromatin immunoprecipitation (ChIP) provides evidence for direct binding of HOXA7 to the hTERT promoter. Current experiments address a possible direct effect of MLL-AF4 on hTERT expression. Furthermore, we are studying the role of HOXA7 and hTERT in MLL-AF4-mediated repression of apoptosis. Conclusions. Our results suggest that MLL-AF4 controls hTERT expression at least in part via HOXA7. The observed changes in methylation pattern of the hTERT promoter upon MLL-AF4 depletion supports a function of this leukemic fusion protein in the epigenetic control of gene expression. Analysis of intracellular signaling pathways may not only elucidate the oncogenic action of MLL-AF4 but may also open the avenue for new treatment options by targeting MLL-AF4 key functional domains. This work was supported by the José Carreras Leukaemia fight (DCLS- R03/10).

0078 PRENYLATION INHIBITORS MODULATE IL-6 AND IGF-1 DEPENDENT SIGNALING IN MULTIPLE MYELOMA CELLS

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Background. Multiple myeloma (MM) is a fatal hematologic malignancy associated with disruption of RAS-to MAP kinase (MAPK/ERK) signaling. IL-6 and IGF-1 promote malignant plasma cell proliferation through stimulation of MAPK and PI-3 kinase/ Akt signaling. Prenylation inhibitors such as farnesyltransferase inhibitors (FTIs), geranylgeranyl transferase inhibitors (GGTIs) and lovastatin block RAS post-translational modification and disrupt RAS signaling. Aims. To assess the efficacy of prenylation inhibitors (e.g. FTI L-744,882, GGTI-2147 and lovastatin) in blocking MM cell responses to IL-6 and IGF-1. Methods. Primary MM cells were isolated by magnetic cell sorting using CD138-coupled microbeads. MM cells were titrated with prenylation inhibitors and IC50’s were determined by proliferation assays (MTS). Synergistic inhibition of MM cell proliferation upon co-treating with FTI and GGTI or lovastatin was evaluated using the CalcuSyn program. Levels of IL-6- and IGF-1-induced phosphorylated MEK-1/2 and MAPK-1/2 were detected by Western blotting. Results. FTI L-744,882 inhibited growth of MM cell line NCI-H929 cultured with IL-6 or IGF-1 even more potently than in cytokine/growth factor-free medium (IC50s 0.1 µM, 1.5 µM and 4.2 µM, respectively). IL-6 and IGF-1 protected NCI-H929 cells from inhibitory effects of GGTI-2147, while IGF-1 had no effect (IC50s 1.1 µM and 0.5 µM vs. 0.5 µM, respectively). IL-6 and IGF-1 protected NCI-H929 cells from lovastatin-induced growth inhibition (IC50s 4.7 µM and 5.0 µM vs. 1.4 µM, respectively). Co-treating NCI-H929 cells with FTI L-744,882 and GGTI-2147 or FTI L-744,882 and lovastatin synergistically inhibited primary MM cell proliferation (IC50’s 0.6-23.1 µM). Activating RAS mutations (4 K-RAS, 1 N-RAS; one sample had both K- and N-RAS mutations) were found in 4/7 (57%) MM patient samples, but anti-myeloma activity of prenylation inhibitors could not be correlated to RAS mutation status. Western blotting demonstrated that FTI/GGTI or FTI/lovastatin co-treatment more completely blocked activation of MEK-1/2 and MAPK-1/2 in NCI-H929 cells than treatment with any of the compounds alone. Furthermore, co-treatment elicited greater inhibition of IL-6 and IGF-1-induced MEK-1/2 and MAPK-2/ activation in NCI-H929 cells. IL-6, IGF-1 and prenylation inhibitors did not affect AKT phosphorylation status in NCI-H929 cells. Summary/Conclusion. Our results support that inhibition of RAS down-stream signaling is a major mechanism through which FTI/GGTI and FTI/lovastatin co-treatment synergistically inhibit MM cell proliferation, even in the presence of IL-6 or IGF-1. In primary MM cells (n=7), FTI L-744,882 elicited anti-myeloma effects only at concentrations much higher than those found to inhibit healthy donor CD34+ cells (IC50’s 51-396 µM vs. 8.2µM) and thus may be ineffective or cause non-specific toxicity when used as a single agent. However, GGTI-2147 and lovastatin induced specific anti-myeloma activity in some cases. Furthermore, combination of FTI with GGTI or lovastatin synergistically inhibited primary MM cell proliferation (IC50’s 0.6-23.1 µM). Activating RAS mutations (4 K-RAS, 1 N-RAS; one sample had both K- and N-RAS mutations) were found in 4/7 (57%) MM patient samples, but anti-myeloma activity of prenylation inhibitors could not be correlated to RAS mutation status. Western blotting demonstrated that FTI/GGTI or FTI/lovastatin co-treatment more completely blocked activation of MEK-1/2 and MAPK-1/2 in NCI-H929 cells than treatment with any of the compounds alone. Furthermore, co-treatment elicited greater inhibition of IL-6 and IGF-1-induced MEK-1/2 and MAPK-2/ activation in NCI-H929 cells. IL-6, IGF-1 and prenylation inhibitors did not affect AKT phosphorylation status in NCI-H929 cells. Summary/Conclusion. Our results suggest that inhibition of RAS down-stream signaling is a major mechanism through which FTI/GGTI and FTI/lovastatin co-treatment synergistically inhibit MM cell proliferation, even in the presence of cytokines and growth factors known to promote MM cell growth (e.g. IL- 6 and IGF-1). Alternative prenylation of K- and N-RAS by GGTAse I in the presence of FTI may explain the clinically observed incomplete response to FTI treatment. As the majority of RAS mutations in multiple myeloma occur in K- and N-RAS, FTI-resistance due to alternative geranylgeranylation may have therapeutic consequences in this disease.
proliferation. Thus, agents that selectively target the survival pathway(s) active in B-CLL cells could reverse drug resistance. Proteasome plays a pivotal role in the control of many apoptotic and cell cycle-regularatory processes, become the focus of new approaches to the treatment of cancer, including B-cell malignancies. Bortezomib is the first proteasome inhibitor approved by FDA and EMEA for the treatment of refractory multiple myeloma. Extensive preclinical data is being developed to study the potential therapeutic of this new drug in other cancers. Our previous results show that BZ induces apoptosis as single agent, in a dose depend-ent manner, showing some selectivity for the transformed cells (EHA, 2005). Our preliminary results support the idea that the Bortezomib apopto-tic effect may occur in a Bax dependent way (ASH, 2005). Here we examined the effects of bortezomib on apoptosis in peripheral blood mononuclear cell isolates from patients with CLL and characterized some of the biochemical mechanisms associated with the response. Mononuclear cells isolated from the blood of 24 CLL patients (14 without and 10 with prior conventional therapy, chlorambucil or fludarabine, 2 of those with refractory disease) were treated in vitro with bortezomib (ranging concentration from 0.1 nM to 10 µM), and evaluated for apoptosis by flow cytometry. Directly conjugated monoclonal antibodies to CD5 and CD19 were used to identify LLC-B cells. The expression of some proteins involved in mitochondria and membrane apoptotic pathways, namely the Bcl-2 proteins family, Bax and Bcl-2, the suppressor protein p53, the IAP survival proteins family and caspase 3 were determined by flow cyntometry using monoclonal antibodies. At 24 h incubation time, bortezomib induces apoptosis in isolated cells from patients without and with prior conven-tional therapy. However an average increase in the percentage of apopto-totic cells versus patients with prior therapy were observed (±25%) which might be associated with a higher Bax expression. Our prevalence results also observed an increase in survivin expression in Bortezomib treated cells which, if confirmed, supports the idea that Bortezomib induces apoptosis by an independent membrane apoptotic pathway. On the other hand, the apoptotic effect seems to be independent of basal TRAIL receptors expression. Our data confirm that bortezomib, like other protea-some inhibitors, has proapoptotic activity in CLL cells. More important-ly, bortezomib was also effective in B-CLL cells isolated from refractory patients. Although the biochemical mechanisms and the extent of this activity and whether or not it translates into clinical benefit will only be known after patients enrolled on a trial have been evaluate.

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0080 NO EVIDENCE FOR CONSTITUTIVELY ACTIVATED FLT3 IN JUVENILE MYELO-MONOCYTIC LEUKEMIA A.C.H. de Vries,1 M.L. den Boer,1 M.M. van den Heuvel-Eibrink,1 R. Pieters,1 F. Schneider,1 R.W. Stam,2 E.R. van Wering,3 C.P. Kratz,1 C.M. Niemeyer,1 O.A. Haas1

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Background. Activating FLT3 mutations have been identified as prognos-tic factors in several myeloid malignancies. Recent studies have demonstra-ted that ligand-independent activation of FLT3 can also result from overex-pression of wild-type FLT3. In addition, ligand-dependent activation has been observed in leukemic cells co-expressing FLT3 ligand (FLT3L), result-ing in autocrine FLT3 signaling which is independent of FLT3 mutations. Aims. In Juvenile Myelo-Monocytic Leukemia (JMML), FLT3 internal tandem duplications (FLT3/ITDs) and mutations affecting the tyrosine kinase domain (TKD) are rare. However, no data are yet available on the frequen-cy of expression of FLT3 and FLT3L in JMML. If activated FLT3 occurs in JMML these patients might benefit from treatment with small molecule FLT3 inhibitors, especially as the curative treatment of JMML is limited to allogeneic stem cell transplantation. Methods. The presence of activating FLT3/ITDs and FLT3/TKD mutations were screened in 51 JMML patients. In 21 patients FLT3 and FLT3L mRNA expression were assessed by real-time quantitative PCR (RT-qPCR) and expression of FLT3 ligand (FLT3L) was measured by FACS. FLT3 and FLT3L expression was determined in 14 patients. Results. In none of the 51 JMML samples FLT3/ITDs or TKD mutations were found. FLT3 appeared to be expressed only at basal levels and FLT3L expression was very low. Consistent with the absence of mutations and lack of FLT3 and FLT3L expres-sion, no PKC 412 cytotoxicity was found in Jak-1/2 sensitive cells with FLT3/ITDs or FLT3L expression. Conclusions. The results showed that constitutive-lly activated FLT3 does not occur in JMML.

0081 MOLECULAR TARGETS OF THE PROTEASOME INHIBITOR, BORTEZOMIB, ON ADULT T-CELL LEUKEMIA CELLS R. Hamamura, J. Ohyashiki, T. Takaku, S. Honda, K. Ohyashiki

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Background. The ubiquitin-proteasome system (UPS) is critical for regu-lation of fundamental cellular systems, such as cell cycle regulation, death, and immune response. The molecular mechanism of proteasome inhibitor-mediated anti-cancer activity has recently been extensively stud-ied, and one of the major pathways is inhibition of the NF-κB cascade. Adult T-cell leukemia (ATL) is a fatal neoplasia derived from HTLV-1 infected T lymphocytes, and NF-κB activation is frequently associated with HTLV-1 infection. Aim. We therefore sought to determine the anti-tumor effect of the proteasome inhibitor, bortezomib in ATL cells, using gene expression profiling. METHODS and Results. Assessment of gene regulation by microarray analysis revealed that down-regulation of genes involved in anti-apoptosis (i.e., BCL2, and IAP5), up-regulation of genes related with apoptosis (i.e., FAF1 and TNFRSF10B), heat shock proteins (i.e., HSPA, HSPCA), and oxygen stress (i.e., heme oxygenase-1). Since heme oxygenase-1 is believed to represent a key enzyme for the protec-tion of cells against stress, it provides a growth advantage and contributes to cellular resistance against chemotherapy. Conclusion. Our results sug-gest that specific inhibition of heme oxygenase-1 expression in combina-tion with proteasome inhibitor may be a new option in treating ATL patients and may be used as a sensitizer for chemotherapy.

0082 ANTISENSE THERAPY AGAINST MULTIDRUG RESISTANT GENES IN ACUTE MYELOBLASTIC LEUKEMIA CELL LINE F. Nадali1, A. Pourfathollah2, K. Ali-moghadam3, A. Dizaji4, A. Zomorodipour5, E. Azizi1, S. Rostami2, A. Ghavamzadeh6

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Background. Acute myeloblastic leukemia (AML) is the most common leukemia in adults. Although the clinical outcome of acute leukemia has been proved by recent progress in chemotherapy, it is still a difficult dis-ease to treat. One major problem is the emergence of leukemic blast cells that are resistant to anticancer drugs. This phenomenon is named mul-tidrug resistance. A representative cause of MDR is the expression of the MDR1 gene and its product, P-glycoprotein (Pgp) on the cell surface mem-brane. Expression of Pgp is associated with its resistance to several types of antineoplastic agents such as anthracyclines, taxans, epipodophylo-toxines and vinca alkaloids. Aim. In this study we tried to reverse MDR phenotype in leukemic cells by antisense in complex to nanoparticle (PEI) against MDR1 gene. Methods. In the present study, the Pgp expressing cell line was established from parental K562 cell line with increasing concen-trations of Doxorubicin starting with 5 ng/mL. The Pgp expressing cell line was obtained in 20 ng/mL and named KDI/20. In order to reverse the MDR phenotype due to Pgp expression four different sequences of sense, antisense and one random sequence with phosphorothioate (PTO) modification (PS-ODN) against MDR1/mRNA was synthesized. They were treated on the KDI/20 in combination with two nonviral vectors: 1)uge 6 transfection reagent (caticonic lipid) and 2) polyelectylenimine (a caticonic polymer, nanoparticle). The effect of PS-ODN was assessed at the cellular level by flowcytometry for Pgp detection, rhodamin 123 assay for functional assessment of Pgp, RT-PCR at the molecular level for MDR1/mRNA and MTT assay in order to assess the sensitivity of cells to Doxorubicin. Results. The results showed a decrease in the percentage of Pgp protein and MDR1/mRNA expression and an increase in the accumula-tion of Rh 123 and drug sensitivity of cells to Doxorubicin by antisense I and III. Summary: The results showed that antisense can reverse MDR phenotype at transcription level and the PEI vector is more efficient than caticonic lipid.
Background and Aims. Systemic mastocytosis is a myeloid neoplasm characterized by abnormal growth and accumulation of mast cells (MC) in visceral organs. In most patients, the D816V-mutated variant of KIT, which mediates resistance against most available tyrosine kinase inhibitors, is found. Therefore current research is focusing on novel targets in MC. We analyzed expression and function of the survival factor Hsp-32 (= heme oxygenase-1, HO-1) in neoplastic MC. Methods & Results. As assessed by Northern blotting and RT-PCR, the human MC line HMC-1 that exhibits KIT D816V, was found to express Hsp-32 mRNA. Expression of the Hsp-32 protein in neoplastic MC was demonstrable by immunocytochemistry and Western blotting. The Hsp-32 inducer hemin (10 µM) increased the expression of Hsp-32 in HMC-1 cells. To examine the role of mutated KIT in expression of Hsp-32, Ba/F3 cells with doxycycline-inducible expression of KIT D816V were employed. In these experiments, KIT D816V was found to induce Hsp-32 promoter activity as well as the expression of Hsp-32 mRNA and the Hsp-32 protein in these cells. The KIT D816V-induced upregulation of Hsp-32 in Ba/F3 cells was completely blocked by a combination of LY294002 (PI3-kinase inhibitor) and PD98059 (MEK inhibitor), but not by single signal transduction inhibitors, suggesting involvement of multiple signaling pathways in KIT D816V-induced Hsp-32 expression. We next examined whether targeting of Hsp-32 is associated with decreased survival. As assessed by 3H-thymidine uptake, the Hsp-32 inhibitor pegylated zinc-protoporphyrin (PEG-ZnPP) reduced the proliferation of HMC-1 cells in a dose-dependent manner (IC50: 5 µM). The PEG-ZnPP-induced inhibition of growth was found to be associated with induction of apoptosis in HMC-1 cells (control: 1±0.6 vs PEG-ZnPP, 5 µM: 55±5% apoptotic cells, p<0.05). Conclusions. Our data show that Hsp-32 is a novel survival factor and interesting target in neoplastic human MC exhibiting the D816V-mutated variant of KIT.

Eph receptors tyrosine kinase and their ephrin ligands, highly expressed during embryogenesis are involved in many key developmental processes. Eph/ephrin interaction triggers a bidirectional transduction cascade that regulates morphogenesis and cell-cell interaction. Although Ephs receptors are not detectable in normal adult tissues, they are overexpressed in many tumors, suggesting a possible role of these PTKs in oncogenesis. Activation of tyrosine kinases and cell-signal transduction pathways are of increasing interest in the pathogenesis of chronic myeloproliferative disorders (CMPD). Dasatinib (BMS-354825), a novel BCR-ABL inhibitor exhibits an interesting inhibitory activity on some tyrosine kinases. Aim: The aim of this study was to comparatively evaluate the expression and function of Ephs and ephrins in CMPD and investigate the possibility of exploiting EphA3 as a therapeutic target of BMS-354825. Methods. EphA3 mRNA expression was analyzed, using Real Time PCR, in 266 samples obtained from CMPD patients (135 PB and 131 BM), 48 with a diagnosis of PV, 55 ET, 20 IM, 24 CMLM, 4 HES, 50 CML in chronic phase and 90 patients with a diagnosis of Ph-CMPD. 38 normal controls (18 PB and 20 BM) were also evaluated. Moreover, we investigated the expression level of EphA3 in 35 sample of B-CLL, 39 AML, 27 ALL and in 7 cell lines (Jurkat, K562, HL-60, MEL, NIH-3T3, 293T, COS-7). Protein expression and localization were examined using Western Blot, Immunoprecipitation and Immunofluorescence analysis with appropriate antibodies. Transient transfection was performed in 293T e COS EphA3- cells using EphA3 plasmid. Nucleotide sequencing of tyrosine kinase catalytic domain was performed in 45 EphA3+ patients and in Jurkat cells. BMS incubation of normal/pathological samples and cell lines was performed (3,10,20 nM). Cells proliferation was evaluated using MTT assay; apoptosis rate was analyzed by FACS (Annexin V) and colony growth was examined on methylcellulose cultures. Results. We found EphA3 overexpression in Ph- mieloproliferative patients (45%) compared to normal controls (5%) (p<0.004 in the PB e p=0.005 in the BM), with a significantly difference in the amount of transcript. 14% of B-CLL, 40% of ALL, 80% of AML and 8% of CML were positive. The overexpression was observed more frequently in BM as compared to PB (51,5% vs 22,8%). No expression difference was noted among the Ph-CMPD. Western Blot analysis confirmed protein expression in EphA3+ samples and revealed receptor phosphorylation. Dasatinib led to significant dose-dependent inhibition of EphA3 phosphorylation. Moreover, BMS induced significant apoptosis (mean value 32%), colony growth reduction (mean value of 54,2 vs 76,5) and proliferation rate inhibition (48%) of EphA3+ cells compared to normal controls. Immunofluorescence assay showed transmembrane localization of EphA3 receptor and revealed cells projections reduction, cell repulsion and cell rounding only in EphA3+ transfected cells. No kinase domain mutations were found in EphA3 overexpressing patients and Jurkat cell lines. Conclusion: EphA3 is abnormally expressed in different hematological malignancies with a significant overexpression in CMPD as compared to normal controls. EphA3 phosphorylation blocking induced by BMS-354825 results in growth arrest and apoptosis of EphA3 over-expressing cells. Therefore, EphA3 may represent a potential candidate for targeted signal transduction therapy.

Background. Mcl-1 is a Bcl-2 family-member that has been described to act anti-apoptotic in various myeloid neoplasms and therefore has been
proposed as a potential therapeutic target. Systemic mastocytosis (SM) is a myeloid neoplasm involving myelomastocytic progenitors. *Aims.* We examined the expression and functional role of Mcl-1 in neoplastic mast cells (MC), to determine whether Mcl-1 could serve as a target in MC neoplasms. *Methods.* As assessed by RT-PCR and immunohistochemistry, primary neoplastic MC were found to express Mcl-1 mRNA and the Mcl-1 protein in a constitutive manner in all patients analyzed. Moreover, the human MC-leukemia cell line HMC-1 was found to express Mcl-1. Transfection of these cells with Mcl-1-specific antisense oligonucleotides (ASO) or an mcl-1-specific siRNA using lipofectin resulted in a reduced survival and increased percentage of apoptotic cells compared to control. *Results.* The effects of mcl-1 ASO were seen with the HMC-1 subclone carrying the G560V c-kit mutation (mcl-1 ASO, 250 nM; 49±4% apoptotic cells compared to control: 3±2%, p<0.05; mcl-1 siRNA: 41±5% vs control: 5±3%, p<0.05) as well as with HMC-1.2 cells carrying both the G560V c-kit mutation and the D816V c-kit mutation (mcl-1 ASO, 250 nM; 56±2% apoptotic cells compared to control: 62±1%, p<0.05; mcl-1 siRNA: 30±6% vs control: 5±2%, p<0.05). Moreover, mcl-1 ASO were found to cooperate with the tyrosine kinase inhibitors (Novartis Pharma AG) imatinib, AMN107, and PKC412 in producing growth inhibition in HMC-1.2 cells. *Summary.* Together, these data show that Mcl-1 is a novel survival factor and attractive target in neoplastic human MC. Whether the Mcl-1-targeting concept can be developed far enough to reach clinical application remains to be elucidated.

### 0086

**ANAGRELIDE: STUDIES ON ITS MODE OF ACTION USING DIFFERENT MODEL SYSTEMS OF MEGAKARYOCYTE DIFFERENTIATION**

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**Background and Aims.** Anagrelide is a potent and selective inhibitor of megakaryopoiesis used for the treatment of essential thrombocythemia. Although the effectiveness of this drug in lowering platelet counts is now firmly established, its primary mechanism of action remains elusive. We have previously demonstrated that anagrelide inhibits the development of megakaryocytes from isolated CD34 positive hematopoietic progenitors (Wang et al. Br. J. Pharmacol. 2005;146:324). Given that the use of cell lines could facilitate the identification of the molecular target of anagrelide, in this study we have compared the effects of the drug on the proliferation and differentiation of CD34 positive cells with those observed in UT7/mpl, a growth factor-dependent haematopoietic cell line engineered to express the human thrombopoietin (TPO) receptor MPL. *Methods.* CD34 positive cells were purified from human umbilical cord blood and cultured for 12 days in IMDM-based medium supplemented with 20 ng/mL TPO. UT7/mpl cells were maintained in exponential growth in α-MEM containing G418 and 2 ng/mL GM-CSF. To induce megakaryocytic differentiation in UT7/mpl cells, GM-CSF was replaced by 20-100 ng/mL TPO; alternatively cells were transfected in the presence of GM-CSF with 10 nM phorbol-myristyl-acetate (PMA). *Results.* Culture of UT7/mpl for 5 days with GM-CSF or TPO led to >10-fold cell expansion. Addition of anagrelide at 1 µM, a concentration which causes maximal inhibition of megakaryocytopenesis in CD34 positive cells and corresponds to >10-fold its IC50 in that system, caused only a slight and non-consistent inhibition of UT7/mpl cell expansion (10%-26% in cells grown with GM-CSF and -8 to +17% in cells grown with TPO). In addition, flow cytometric analysis showed that, unlike its effect in CD34 positive cell cultures, in UT7/mpl cells anagrelide did not inhibit TPO-induced expression of the megakaryocytic differentiation marker CD61. Furthermore, the lack of anagrelide activity was unrelated to the concentration of TPO used. Since UT7/mpl cells undergo megakaryocytic differentiation also when treated with phorbol esters, the activity of anagrelide against this class of agents was also tested. PMA completely inhibited UT7/mpl cell growth, caused a marked enlargement in cell size and induced a >3-fold increase in the expression of CD61. Addition of 1 µM anagrelide had no significant effect on any of these parameters. *Conclusions.* These findings indicate that UT7/mpl cells cannot replace normal hematopoietic progenitors as an in vitro model system to study the mechanism by which therapeutic doses of anagrelide inhibit megakaryocytic differentiation. In addition, our study suggests that the molecular target of anagrelide lies further down stream from the ligand binding site of MPL. Since PMA induces megakaryocytic differentiation through activation of protein kinase C, our study further suggests that this pathway is not a target of anagrelide.

### 0087

**TISSUE-PLASMINOGEN ACTIVATOR AND PLASMINOGEN ACTIVATOR INHIBITOR IN ESSENTIAL THROMBOCYTHEMIA AND POLYCYTHEMIA VERA**

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A hypercoagulability state exits in patients with essential thrombocythemia (ET) and polycythemia vera (PV). *in vitro* and *in vivo* data demonstrated a reactive release from vascular wall of tissue plasminogen activator (t-PA) and plasminogen activator-inhibitor-1 (PAI-1) after increase in thrombin and t-PA concentration, respectively. Elevated plasma levels of t-PA and PAI-1 were found in patients with ET and PV. However, few studies have concentrated on the correlation between hypercoagulability and fibrinolytic system in these malignancies. Prothrombin fragment F1+2 (F1+2), marker of thrombin generation, t-PA, activation marker of fibrinolysis, PAI-1 specific plasminogen activator inhibitor and d-dimer (DD), a product of fibrinolysis, were investigated in patients with ET and PV. As PAI-1 is abundant in platelet α-granules, we also measured b-thromboglobulin (b-TG) and platelet factor 4 (PF4) and platelets. We included 44 patients, 22 ET (5 men and 17 women, mean age 55 years) and 22 PV (17 men and 5 women, mean age 60 years) who fulfilled PVSG. The mean duration of disease was 4.5 years. Of 44 patients, 27 (13 ET, 14 PV) received hydroxyurea, 2 ET were on interferon-α, 5 ET were on anagrelide hydrochloride, 8 PV underwent phlebotomy and 2 ET not receiving any cytoreduction. All patients were on antiplatelets. None of studied patients had thrombotic risk factors. t-PA, PAI-1, F1+2, b-TG and PF4 were assayed by ELISA and DD by immunnoassay. Platelet aggregation. Platelets were determined byautomated analyser. F1+2 (2.3±2.8 nmol/L vs 0.7±0.2 nmol/L) (*p*<0.001) was increased as well as t-PA and PAI-1 (112±67 ng/mL and 50±24 ng/mL, respectively, vs. 9.6±2.4 ng/mL and 24±9 ng/mL, respectively) (*p*=0.0001 and *p*=0.0001, respectively), whereas DD was normal (157±125 ng/mL). All patients had elevated b-TG and PF4 (352±608 IU/mL and 129±49 IU/mL, respectively vs 28±2 IU/mL and 5±2 IU/mL, respectively) (*p*=0.0001 and *p*=0.0001, respectively) and normal platelets (402±148±10^4^/L). We found a correlation between F1+2 and t-PA (0.0001) and an association between t-PA and PAI-1 (0.0001). No correlation was between PAI-1 and b-TG and PF4 and platelets. Our findings suggest a relationship between hypercoagulability and hypofibrinolysis as reflected by high PAI-1 and normal DD. Additionally, it is hypothesized that increased PAI-1 is independent of platelet abnormalities suggesting that a vasculopathic hypofibrinolysis may be in patients with ET and PV.

### 0088

**PLATELET, LEUKOCYTE AND COAGULATION ACTIVATION PATTERNS IN MYELOFIBROSIS WITH MYELOID METAPLASIA**


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**Background.** Platelet and leukocyte activation has been demonstrated in polycythemia vera (PV) and essential thrombocythemia (ET), this being considered as a contributory mechanism to thrombosis. Data in patients with myelofibrosis with myeloid metaplasia (MMM) are, however, scarce. *Aims.* To assess the status of platelet, leukocyte and coagulation activation in patients with MMM. *Methods.* Platelet and leukocyte activation status (measured at baseline and after ADP, thrombin and arachidonic acid stimulation), platelet-neutrophil and platelet-monocyte complexes, and CD11b determination in the neutrophils and monocytes, was assessed by flow cytometry in 20 MMM patients and in 22 age- and sex-matched healthy individuals. JAK2 V617F mutational status and the markers of coagulation activation were also assessed in 15 MMM patients. *Results.* In MMM patients the μCD62P, μCD63, μCD42d and μCD61 expression (measured at baseline and after ADP, thrombin and arachidonic acid stimulation), platelet-neutrophil and platelet-monocyte complexes, and CD11b determination in the neutrophils and monocytes, was assessed by flow cytometry in 20 MMM patients and in 22 age- and sex-matched healthy individuals. JAK2 V617F mutational status and the markers of coagulation activation were also assessed in 15 MMM patients.

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No differences in platelet and leukocyte activation were observed according to t(9;22) (Philadelphia chromosome). Patients with myelophthisis show platelet, leukocyte and coagulation activation patterns similar to those found in PV and ET.

**0089**

**EPIDEMIC ALTERATIONS AND MUTATION OF JAK2 TYROSINE KINASE IN PATIENTS WITH BCR/ABL NEGATIVE MYELOPROLIFERATIVE DISORDERS**

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Background. Bcr/abl negative myeloproliferative disorders (MPD) are a group of clonal stem cell diseases and comprise traditionally essential thrombocythemia (ET), polycythemia vera (PV) and myelofibrosis with myeloid metaplasia (MfM). The recent discovery of the autoactivating mutation with a V617F amino acid substitution in the JAK2 tyrosine kinase domain has been a great step towards understanding of the pathophysiology of MPD. However, this mutation is found only in about half of the patients with MPD. Aims. Hypermethylation of CpG islands within gene promoter associated regions is associated with transcriptional inactivation and represents an important mechanism of gene silencing in the pathogenesis of human cancer. In this study, we sought to determine the potential role of DNA methylation changes in the context of JAK2 mutation in MPD. Methods. We analysed the JAK2 mutational status by direct sequencing and the methylation patterns of 12 cancer-related genes by methylation specific polymerase chain reaction in bone marrow and blood specimens from 35 patients with MPD. Genes analysed were SOCS-1, E-cadherin, MGMT, TIMP-2, TIMP-3, p15, p16, p53, DAPK1, RASSF1, RAR-2 and MLH1. Results. The frequency of aberrant methylation was 4/22 for SOCS-1, 1/22 for p15, TIMP-2 and E-cad in patients with MM, 1/7 for SOCS-1 and MGMT in PV and 1/4 for SOCS-1 and DAPK1 in ET. We detected at least one hypermethylated gene in 11/35 patient samples. The JAK2V617F mutation was found in 9/22 patients with MPD, 4/7 in PV and 4/4 with ET. Our data indicate that hypermethylation of tumour suppressor genes can be considered as a common phenomenon in bcr/abl negative MPD in addition to the JAK2V617F mutation. We found concomitant heterozygous mutation of JAK2 and hypermethylation of the cytokine regulator SOCS-1 in two patients. The cell adhesion gene E-cadherin was methylated in one patient with MM in 11/35 patient samples. In the patient with PV was detected to carry both mutation of JAK2 and hypermethylation of SOCS-1. However, in most patient samples, we found either JAK2V617F somatic mutation without CpG island hypermethylation or altered methylation patterns without genetic aberration of JAK2. In conclusion, in addition to the recently discovered activating mutation of JAK2, CpG island hypermethylation of cancer-related genes, especially SOCS-1, a negative regulator of JAK2. These results suggest, that epigenetic changes may, in addition to the well defined JAK2 activating mutation, contribute to the pathogenesis of bcr/abl negative MPD and thus can be considered as a potential therapeutic target for demethylating agents.

**0090**

**CLINICOPATHOLOGICAL HETEROGENEITY OF CHRONIC HYPEREOSINOPHILIC SYNDROMES LONG-TERM EXPERIENCE ON 32 PATIENTS**


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Background. Chronic hypereosinophilic syndromes (CHS) comprise a wide spectrum of indolent to aggressive diseases characterized by prolonged, unexplained hypereosinophilia. Aims. A study was planned to evaluate clinical and pathologic features in patients with chronic, non-reactive hypereosinophilia. Material: 32 patients (pts) observed between 1990-2005 with absolute eosinophilia count (AEC) of higher than 0.7×109/L for at least 5 months were included. Results. There were 13 males and 19 females, aged 18-76 years (median 52 yrs). The diagnosis was following: Hypereosinophilic Syndrome (HES, n=16), Chronic Idiopathic Eosinophilia (CIE, n=12), T-cell mediated HES (n=1), Chronic Eosinophilic Leukemia (CEL, n=1) and CEL PDGFRα+ (n=2). Organ involvement included: heart (n=9), lungs (n=5), spleen (n=7), liver (n=4), lymph nodes (n=5), skin (n=3), peripheral nerves (n=2) and gut (n=1). Median white blood cell (WBC) count at diagnosis was 12.6×109/L (range 5.2-81.6), with AEC of 4.32×109/L (0.9-32.9) and bone marrow eosinophilic infiltration of 30% (8.0-55.0). Median IgE level was 95.2 IU (range 0.1-13966), vitamin B12 concentration-250pg/ml (range 60-3559). Only one patient revealed a slight increase in spindle-shaped mast cells on bone marrow exam, but there were no c-Kit+ and FIP1L1-PDGFRA mutations in this case. On cytogenetic evaluation normal karyotype was present in 13/14. One patient with CDD+CD8+ T-cells and TCRβ rearrangement showed t(6;11)(p21;q23). BCR/ABL was undetectable in 19/19. RT-PCR for FIP1L1-PDGFRA was detectable in 2 of 19 pts at diagnosis (11%). The first-line therapy consisted of steroids and hydroxyurea. In 21, patient with T-cell mediated HES received CHOP regimen and one patient with CEL in accelerated phase was given induction therapy (HA- 
hydroxyurea, adriamycin, ara-c). Majori of pts with HES and CIE responded promptly to low dose of prednisons (10-20 mg/day), but eosinophilia 
recurred shortly after prednisons tapering or discontinuation. Eight patients due to resistance to prior therapy were administered imatinib at initial dose of 100 mg daily. A complete remission was documented in 3/8 (37%), Two out of three, who achieved complete hematological remission in a median time of 14 days (range 13-85), were FIP1L1-PDGFRA positive at diagnosis. One out of two FIP positive patients responded to hydroxyurea and cytarabine at six months. Remaining patients attempted to discontinue imatinib, but relapsed promptly. Imatinib was resumed at 100 mg daily with rapid eosinophilia resolution. Patient with CEL underwent allogeneic bone marrow transplantation from his brother and currently is disease-free. Patient with T-cell mediated HES was pretreated with hydroxyurea and allogeneic stem cell transplantation with autologous stem cell collection after complete molecular remission while eosinophilia persisted. One patient developed pure red cell aplasia during the disease course and it results from the prior hydroxyurea treatment. Current status of pts included to the study is; complete remission in 17 pts, partial response 4 pts, non-responders- 10 pts, 1 death due to cardiac insufficiency. Conclusions. Our study showed that majority of pts with CHS has a benign disease course and steroids are sufficient to control the eosinophil count. We confirmed the high efficacy of low dose of imatinib in patients carrying the FIP1L1-PDGFRA mutation. Discontinuation both steroids and imatinib was followed by rapid eosinophilia recurrence.

**0091**

**APPLICATION OF PRV-1 MRNA EXPRESSION LEVEL AND JAK2V617F MUTATION FOR DIFFERENTIAL DIAGNOSTICS BETWEEN POLYCYTHEMIA VERA AND SECONDARY ERYTHROCYTOSIS. INFLUENCE OF INTERFERON THERAPY ON PRV-1 EXPRESSION**


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Background. Polycythemia vera (PV) is a clonal myeloproliferative disorder (MPD) lacking specific biological markers. Recent discovery of PV and JAK2V617F overexpression and Jak2V617F mutation in the majority of patients with PV can facilitate differential diagnosis between PV and non-malignant disease - Secondary Erythrocytosis (SE). Furthermore, recently established influence of interferon (IFN) therapy on PRV-1 overexpression might reflect treatment efficiency. Aims. Confirm PRV-1 mRNA overexpression and Jak2V617F mutation in the group of patients diagnosed with PV and absence of these markers in the group of patients diagnosed with SE. Investigate influence of Interferon therapy on PRV-1 expression levels in patients with PV. Methods. We studied 46 patients (22 M/24 F) diagnosed with polycythemia vera based on PVSG criteria. For 39 patients diagnosis was confirmed by histological studies. Average patient age was 54 years, average time from diagnosis was 6.8 years. At the time of study out of 46 patients 9 were naive and did not receive cytostatic treatment, 25 were pretreated with Hydroxyurea (HU) and 12 were pretreated with IFN. PRV-1 expression level was determined twice for 7 patients continuously receiving 5 millions ME daily 8 months after first analysis. Also we studied 14 patients with secondary erythrocytosis. Total duration of Interferon treatment for these patients was 23 months. Control group includes fifteen normal donors. PRV-1 expression level was determined by reverse transcription and quantitative PCR (iCycler IQ, BioRad). Normalization to β2 microglobulin expression level was used for comparison between different samples. Jak2V617F mutation was determined by PCR-RFLP and real-time PCR. Results. In the control group PRV-1 mRNA overexpression was observed in 8 out of 9 naive patients without cytostatic treatment, in 21 out of 25 patients pretreated with HU and in 8 out of 12 patients
pretreated with IFN. Overall we found PRV-1 overexpression in 37 out of 46 patients in the study (80%). We did not find PRV-1 overexpression in patients diagnosed with SE (Figure, left panel). Sequencing for determination of Jak-2 mutations was performed for 22 patients. A total of 10 patients presented with SE and healthy donors were examined. Mutation was found in all patients with PV and was not found in patients with SE and healthy donors. After 8 months of prolonging treatment with IFN, PRV-1 overexpression level was decreased strongly (p=0.04) in all seven examined patients (Figure, right panel). Six patients from this group achieved remission at the time of second analysis. Criteria’s of remission were reduction of platelet level down to 400-600×10^9/L, leukocytes to 10-12×10^9/L, and erythrocytes to 6×10^12/L.

Conclusions. We show high sensitivity specificity and utility of PRV-1 expression level and especially Jak2V617F mutation for differential diagnosis between PV and SE. Decrease of prv-1 expression levels in the group of patients receiving Interferon might be designated in the future as a molecular marker of treatment efficiency.
PREVIOUS HISTORY OF THROMBOTIC COMPLICATION IS THE MAIN RISK FACTOR THAT INCREASES THE INCIDENCE OF THROMBOTIC EVENTS IN ESSENTIAL THROMBOCYTHEMIA PATIENTS

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Background and Aims. Thrombotic and hemorrhagic complications are the main causes of morbidity in essential thrombocythemia (ET). We investigated the clinical and laboratory characteristics associated with the occurrence of these events, with the aim of identifying subgroups of patients who would benefit from antithrombotic and/or anticoagulant therapy. Methods. 306 consecutive ET patients followed between January 1997 and December 2002 (median age 58 years, male/female ratio 0.55, median follow-up 96 months) were included in this study. In order to identify the possible predictive factors of thrombotic risk, the following variables were considered: age, gender, platelet count at diagnosis and at the time of thrombotic event, previous history of thrombotic and/or hemorrhagic complications, disease duration and cardiovascular risk factors (arterial hypertension, hypercholesterolemia, diabetes, smoking, obesity and a familial history of thrombosis). Results. 46 patients (15%) experienced thrombotic complications during the course of disease. Major thrombotic complications included stroke, transient ischemic attack, myocardial infarction, angina pectoris, peripheral arterial thrombosis, retinal artery occlusion, deep venous thrombosis and pulmonary embolism. Age, gender, platelet count at diagnosis and at the time of thrombotic event, and disease duration did not appear in our series to significantly increase the incidence of thrombotic complications. These events occurred in 26/64 (40.6%) patients with a previous history of thrombosis and in 20/242 (8.3%) without a previous history of thrombosis (p<0.001 Fisher's exact test, odds ratio 7.6). When patients with no previous history of thrombosis were stratified according to the number of cardiovascular risk factors (arterial hypertension, hypercholesterolemia, diabetes, smoking, obesity and a familial history of thrombosis), we observed a significant correlation with the occurrence of thrombotic events (p<0.05). 31 patients (10%) experienced major hemorrhagic complications, mainly gastrointestinal tract bleeding; three of them had a positive and 28 a negative history of hemorrhagic events (p=0.052). Major bleeding was defined as an event that threatened life or organ function, or required a transfusion of red blood cells. Conclusions. This study was based on a large cohort of patients followed for many years at a single institution and confirmed that a previous history of thrombosis is the main risk factor for developing further thrombotic events during the follow up. Age and platelet count, generally accepted as very important risk factors for thrombosis, did not appear in our series associated with an increased risk for thrombosis. Asymptomatic patients with a negative history of thrombosis and without any cardiovascular risk factors can be considered at low risk and therefore should not be considered for treatment - regardless of platelet count, age, sex and the disease duration. For patients with a negative history of thrombosis, but at high cardiovascular risk, it is essentially associated with them and further evaluation of the opportuneness of an antiaggregant and/or cytoresponsive therapy.

RATIONAL AND DESIGN OF A DOUBLE-BLIND, PLACEBO-CONTROLLED TRIAL TO EVALUATE THE CORTICOSTEROID-SPARING EFFECTS OF ANTI-ILS MONOCLONAL ANTIBODY (MEPOLIZUMAB) IN SUBJECTS WITH HYPEREOSINOPHILIC SYNDROME

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Background. Hypereosinophilic syndrome (HES) comprises a group of rare hematological disorders characterized by sustained eosinophil overproduction. Clinical manifestations result from damage to multiple organs associated with local release of toxic granule products by infiltrating eosinophils. Management is currently based on corticosteroid, interferon-α, and/or cytotoxic therapies, each of which is associated with significant toxicity and tolerability issues. Mepolizumab is a humanized anti-IL-5 monoclonal antibody that blocks the actions of IL-5, the major hematopoietin responsible for eosinophil production, differentiation, and survival. Preliminary data from a small number of patients with HES, asthma, and eosinophilic dermatitis treated with intravenous mepolizumab was associated with reduced blood eosinophils and was well-tolerated. Due to the variability in clinical presentation and lack of validated disease indices for HES, clinical parameters alone may not provide a sensitive and precise measure of drug efficacy. However, the ability of a treatment to enable corticosteroid avoidance could be an important endpoint. Here we describe the design and conduct of a trial currently underway to evaluate the steroid-sparing effects of mepolizumab treatment in patients with HES. Aims. The primary objective of this ongoing study is to assess the effect of mepolizumab versus placebo on reducing corticosteroid requirements in patients with HES requiring 20-60 mg/day of prednisone to maintain eosinophils at <1500cells/µL. The primary endpoint is the proportion of subjects requiring ≤10 mg/day prednisone for at least 8 consecutive weeks during the 32 week treatment period. Methods. This multicentre (30 sites worldwide), randomized, double-blind, placebo-controlled, parallel-group study recruited patients 18-85 years of age with active HES (blood eosinophil count >1500 cells/µL) of ≥26 weeks duration with evidence of organ involvement or dysfunction related to eosinophilia, without any other cause of eosinophilia, who were steroid-responsive and tested negative for the FIP1L1-PDGFRA gene rearrangement. Eligible patients were stabilized on prednisone monotherapy (20-60 mg/day) for ≥3 months, then randomised to receive intravenous mepolizumab 750 mg or saline (placebo) every 4 weeks. Prednisone was tapered at weekly intervals following the first infusion according to a pre-specified algorithm. HES-related end-organ involvement was monitored using cutaneous assessments, echocardiograms, computed tomography scans of the lung, abdomen and maxillary sinus, pulmonary function tests, and esophagogastroduodenoscopy. Patients' perceptions of HES symptom bother, health status, and limitations of daily living were determined using quality of life questionnaires. Blood samples were collected to characterize mepolizumab pharmacokinetics. Subjects completing the trial, or who withdrew due to lack of efficacy, could enter an open-label extension study to evaluate the long-term safety, efficacy and optimal dosing frequency of intravenous mepolizumab. Results. This trial, initiated in March 2004, was fully enrolled by May 2005 with 85 patients started on study medication. Summary/Conclusions. This ongoing study is the largest trial to be conducted to date in patients with HES, and the only placebo-controlled trial in this population. From this study we will derive the most important information on the treatment of HES with mepolizumab, and enable better understanding of this rare condition.

FIPI1L-PDGFRA+ HYPEREOSINOPHILIA: HOW MANY DISEASES?

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Background. The Hyper eosinophilic Syndrome (HES) has remained for a long time a problematic diagnosis. WHO criteria relies on identification of rare signs of clonality which allow differential diagnosis between chronic eosinophilic leukaemia (CEL) and true idiopathic states (HES). Recently, a new mechanism of mutation was described: a cryptic interstitial microdeletion at chromosome band 4q12 generating a FIP1L1-PDGFRA (FP) fusion gene (FG). According to the WHO guidelines, this clonal abnormality has been proposed as a new surrogate marker for CEL. Subsequently, the F/FP FG was reported in patients with hypereosinophilia and atypical bone marrow (BM) mast cells (MC), suggesting a new hypothetical systemic mast cell disorder with hypereosinophilia subgroup (FP+ SMCD-eos). Unfortunately, these SMCD-eos diagnoses were mainly based on histologic criteria (i.e., the major and the first minor WHO criteria) which are essentially subjective. The leukemic stem cell where the FG deletion arises as well as the specificity of the loosely aggregates of tryptase positive mast cells in CEL remain to be identified before the relationship between these two clinical entities could be deciphered. In this regard, WHO guidelines for SMCD-eos differential diagnosis may deserve to be updated. Aims. We stressed out the potential subjective bias in WHO criteria interpretation and questioned the relationship between FIPI1L-PDGFRA+ CEL and SMCD-eos.
Specificity of tryptase expression in the context of hypereosinophilic diseases was assessed at the protein and mRNA level.

Table 1. Clinical, cytogenetics and molecular characteristics of the patients.

<table>
<thead>
<tr>
<th>Cases</th>
<th>Patient # 1</th>
<th>Patient # 2</th>
<th>Patient # 3</th>
<th>Patient # 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>39M</td>
<td>73M</td>
<td>41M</td>
<td>40M</td>
</tr>
<tr>
<td>Dominant symptoms</td>
<td>Thoracic and back pain</td>
<td>Lab discovery</td>
<td>Cough, weight loss, asthenia</td>
<td>Cough, dyspnea, weight loss, fatigue</td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Cardiac involvement</td>
<td>Mitral valve disease</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Other organ involvement</td>
<td>Pulmonary</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Therapy before indolent phase</td>
<td>Steroids</td>
<td>Steroids</td>
<td>Steroids, HU, IFN</td>
<td>Steroids, HU, IFN</td>
</tr>
<tr>
<td>Serum V10 12 (µg/mL)</td>
<td>&gt;3000</td>
<td>845</td>
<td>&gt;2000</td>
<td>&gt;2000</td>
</tr>
<tr>
<td>Serum IgG (µg/mL)</td>
<td>14.1</td>
<td>47.9</td>
<td>22</td>
<td>N.A.</td>
</tr>
<tr>
<td>Serum IgM</td>
<td>10.2</td>
<td>9.4</td>
<td>15.1</td>
<td>12.6*</td>
</tr>
<tr>
<td>Serum IgA</td>
<td>6.5</td>
<td>10.4</td>
<td>10.1</td>
<td>12.6*</td>
</tr>
<tr>
<td>C-reactive protein (µg/dL)</td>
<td>15.8</td>
<td>12.7</td>
<td>58.1</td>
<td>27.3*</td>
</tr>
<tr>
<td>Platelet (&gt;10^9/L)</td>
<td>194</td>
<td>354</td>
<td>27</td>
<td>116*</td>
</tr>
<tr>
<td>BM blasts (%)</td>
<td>&lt;5</td>
<td>2</td>
<td>&lt;5</td>
<td>1.3*</td>
</tr>
<tr>
<td>BM eosinophils (%)</td>
<td>40</td>
<td>19</td>
<td>38</td>
<td>9**</td>
</tr>
<tr>
<td>Myeloid blasts</td>
<td>-</td>
<td>N.D.</td>
<td>-</td>
<td>***</td>
</tr>
<tr>
<td>BM tryptase (+/--)</td>
<td>-</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>Multifocal/oligoclonal infiltrates</td>
<td>-</td>
<td>N.D.</td>
<td>-</td>
<td>***</td>
</tr>
<tr>
<td>Bone marrow involvement</td>
<td>-</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>TCRgamma gene rearrangement</td>
<td>N.A.</td>
<td>N.A.</td>
<td>Polyclonal</td>
<td>N.D.</td>
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<tr>
<td>Karyotype</td>
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<td>46,XY (21)</td>
<td>46,XX (42)</td>
<td>46,XY (42)</td>
</tr>
<tr>
<td>Therapy before imatinib</td>
<td>Steroids</td>
<td>Steroids</td>
<td>NO</td>
<td>Steroids, HU, IFN</td>
</tr>
<tr>
<td>Other organ involvement</td>
<td>Pulmonary</td>
<td>NO</td>
<td>Pulmonary</td>
<td>NO</td>
</tr>
<tr>
<td>Other organ involvement</td>
<td>Pulmonary</td>
<td>NO</td>
<td>Pulmonary</td>
<td>NO</td>
</tr>
<tr>
<td>Serum Vit B12 (pg/mL)</td>
<td>&gt;2000</td>
<td>&gt;2000</td>
<td>&gt;2000</td>
<td>&gt;2000</td>
</tr>
<tr>
<td>ANC (10^9/L)</td>
<td>109</td>
<td>15.8</td>
<td>12.7</td>
<td>58.1</td>
</tr>
<tr>
<td>AEC (10^9/L)</td>
<td>194</td>
<td>314</td>
<td>27</td>
<td>116*</td>
</tr>
</tbody>
</table>

Methods. We described four patients initially diagnosed with HES according to the WHO guidelines, draw special attention to the disease clinico-biological characteristics and highlighted the WHO guidelines ambiguities. Tryptase expression was further assessed at the protein level using specific immunostaining or hemotoxylin-eosin coloration. The F/P fusion gene was identified by FISH and RT-PCR in all our patients. Tryptase mRNA expression was not increased in patients compared with controls. Serum VEGF levels were correlated with the percentage of bone marrow VEGF positive cells in all patients (r=0.58 p=0.001), as well as in MMM and ET patients separately compared with control group (r=0.68 p=0.001 and r=0.58 p=0.001 respectively). Serum VEGF levels were also correlated with MVD in all patients and in MMM patients separately (r=0.4 p=0.0013 and r=0.58 p=0.007 respectively). Bone marrow VEGF expression was not correlated with MVD. Conclusions. These data suggest a model during the course of cMPD, in which amplified secretion of VEGF from bone marrow cells and not increased numbers of VEGF secreting cells, produce increased levels of VEGF contributing to the disease phenotype. Anti-VEGF therapies may have a role in cMPD.

Background. Essential thrombocythemia (ET) is a chronic myeloproliferative disorder characterized by a hyperplasia of the megakaryocytes cells in bone marrow resulting in a persistent increase of the platelet number in peripheral blood. Only about 5% of ET patients show chromosomally abnormal clones by conventional cytogenetics (CC), and about 15% of cases present chromosomal aberrations when FISH probes such as centromeric probes for chr4, 5, 8, 10, 11, 13, and 22 were applied. By contrast, myelocytes and megakaryocytes exhibited a variable expression of VEGF protein only in patients. Osteoblasts, osteoclasts and fibroblasts also were labeled by the anti-VEGF antibody in MMM patients. Serum VEGF levels were correlated with the percentage of bone marrow VEGF positive cells in all patients (r=0.58 p=0.001), as well as in MMM and ET patients separately compared with control group (r=0.68 p=0.001 and r=0.58 p=0.001 respectively). Serum VEGF levels were also correlated with MVD in all patients and in MMM patients separately (r=0.4 p=0.0013 and r=0.58 p=0.007 respectively). Bone marrow VEGF expression was not correlated with MVD. Conclusions. These data suggest a model during the course of cMPD, in which amplified secretion of VEGF from bone marrow cells and not increased numbers of VEGF secreting cells, produce increased levels of VEGF contributing to the disease phenotype. Anti-VEGF therapies may have a role in cMPD.

**0097**

VEGF SERUM LEVELS AND VEGF BONE MARROW IMMUNOHISTOCHEMICAL EXPRESSION IN CHRONIC MYELOPROLIFERATIVE DISORDERS

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Background. Angiogenesis plays a significant role in the pathogenesis and progression of chronic myeloproliferative diseases (cMPD). Vascular endothelial growth factor (VEGF), the most potent direct-acting angiogenic factor known, has been identified as a major cytokine underlying pathological angiogenesis and hematopoiesis. Aims. To evaluate VEGF serum levels and VEGF bone marrow immunohistochemical expression in cMPD patients. To further analyze for possible correlations, between VEGF serum levels and other histopathological parameters, including VEGF immunohistochemical expression and bone marrow microvessel density (MVD). Methods. We evaluated serum levels of vascular endothelial growth factor (VEGF) in 75 patients with cMPD (25 patients with myelofibrosis with myeloid metaplasia (MMM), 40 with essential thrombocythaemia (ET) and 8 with polycythaemia vera (PV)). Twenty seven healthy subjects' age and sex matched to the patient cohort were also included in the study. Moreover, immunohistochemical expression in bone marrow specimens in 25 MMM and 36 ET patients was studied. Results. We found that serum VEGF levels were significantly increased in cMPD patients comparing to controls (all p values ≤0.012). Interestingly, bone marrow VEGF immunohistochemical expression was not increased in patients compared with controls. A high level of VEGF protein was detected in erythroid cells in patients and controls by contrast myelocytes and megakaryocytes exhibited a variable expression of VEGF protein only in patients. Osteoblasts, osteoclasts and fibroblasts also were labeled by the anti-VEGF antibody in MMM patients. Serum VEGF levels were correlated with the percentage of bone marrow VEGF positive cells in all patients (r=0.58 p=0.001), as well as in MMM and ET patients separately compared with control group (r=0.68 p=0.001 and r=0.58 p=0.001 respectively). Serum VEGF levels were also correlated with MVD in all patients and in MMM patients separately (r=0.4 p=0.0013 and r=0.58 p=0.007 respectively). Bone marrow VEGF expression was not correlated with MVD. Conclusions. These data suggest a model during the course of cMPD, in which amplified secretion of VEGF from bone marrow cells and not increased numbers of VEGF secreting cells, produce increased levels of VEGF contributing to the disease phenotype. Anti-VEGF therapies may have a role in cMPD.

**0098**

ARRAY COMPARATIVE GENOMIC HYBRIDIZATION REVEALS AN ABSENCE OF RECURRENT GENOMIC COPY NUMBER CHANGES IN ESSENTIAL THROMBOCYTHEMIA


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Background. Essential thrombocythemia (ET) is a chronic myeloproliferative disorder characterized by an hyperplasia of the megakaryocytes cells in bone marrow resulting in a persistent increase of the platelet number in peripheral blood. Only about 5% of ET patients show chromosomally abnormal clones by conventional cytogenetics (CC), and about 15% of cases present chromosomal aberrations when FISH probes such as centromeric probes for chromosomes 8 and 9, and locus specific probes for 18q14 and 20q12 regions are applied. Thinking on the possibility of the existence of small DNA gains or loses not detected by the conventional cytogenetic analysis, a large number in peripheral blood. Only about 5% of ET patients show chromosomally abnormal clones by conventional cytogenetics (CC), and about 15% of cases present chromosomal aberrations when FISH probes such as centromeric probes for chromosomes 8 and 9, and locus specific probes for 18q14 and 20q12 regions are applied. Four cases of all the genome analyzed by FISH, array comparative genomic hybridization (aCGH) appeared to be a potentially useful new technology. Aims. The aim of the present study was to perform aCGH in order to identify at high resolution regions of DNA copy number changes associated to ET. Patients and Methods. Twenty cases diagnosed of ET according to the PVSG criteria (1997) and who had never received cytolytic treatment were studied. Conventional cytogenetics was performed in 17 cases, and 13 of them showed a normal karyotype while in 4 cases no metaphases were available. In five cases, FISH with BAC probes for the PRV-1, TPO and c-MPL genes was done, but no genetic abnormalities were detected. Genomic DNA was extracted from fresh frozen granulocytes, and pools of karyotypically normal males and females DNAs were used as controls. Forward and reverse hybridiza-
tions were performed in test and control samples using the Spectral Chip 2600TM (Spectral Genomics), an array consisting on 2,621 BAC clones at an average of 1-2 Mbp resolution, according the manufacturer’s specifications. Fluorescent images were obtained using an Agilent G2565BA scanner and quantified using GenePix 6.0 software (Axon, Molecular Devices) using the irregular feature finding option. Extracted raw data was filtered and normalized using Bacanal (Lozano et al., unpublished), an in house web implementation of the Limma package developed within the Biocductor project in the R statistical programming environment. Results. Among the 20 analyzed patients, in two cases a genomic copy number change was detected. Case 2 showed a gain of 3p24-p24.3 (RP11-245E5, RP11-208G16) and case 6 presented a gain of 8p23.2 (RP11-121H7, RP11-11H7), a region that encodes the CSMD1 gene. In addition, two patients (cases 11 and 12) showed variation copy number polymorphisms in 16p11.1-p11.2 (RP11-48820, variation 0196 and RP11-80F22, variation 0197) and in 2q37.3 (RF5-1011017, variation 0052 where FLI4072 and FLJ41327 are located and CTR-17211), respectively. The gains and losses detected in these patients were not detected in the remaining patients. Comments. Array CGH reveals an absence of recurrent genomic copy number changes in essential thrombocythemia. FISH studies with the affected BAC clones will be performed to confirm these Results. Acknowledgments. Grants FIS PI030845, C08/07 and C03/10 from the Spanish Ministry of Health.

0099

THE INCIDENCE OF DEL20Q12 BY INTERPHASE FLUORESCENCE IN SITU HYBRIDIZATION (I-FISH) IN PATIENTS WITH CHRONIC MYELOPROLIFERATIVE DISORDERS

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Background. Chronic Myeloproliferative Disorders (CMPD), including Polycythemia Vera (PV), Essential Thrombocythemia (ET), Idiopathic Myelofibrosis (IMF) and Unclassified CMPD, are acquired diseases of the hematopoietic stem cell, characterized by clonal proliferation of one or more cell lines. Chromosomal abnormalities have been reported in less than 20% of CMPD patients at the time of diagnosis and in higher frequencies during the course of the disease.

Aim. The aim of the present study was to determine by I-FISH the incidence of 20q12 deletion (del20q12) (D20S108) in bone marrow samples from CMPD patients either at diagnosis or during the course of the disease and to assess its clinical utility.

Patients and Methods. Eighty four samples from 38 men and 40 women with median age 63 (range 17-84) years were studied using I-FISH, utilizing a locus specific probe for 20q12 (D20S108). 38 patients were diagnosed with ET, 34 with PV, 3 with IMF and 3 with unclassified CMPD.

Bone marrow samples from 12 healthy volunteers were used as assay validation controls. 31 samples were studied at diagnosis, whereas 53 followed the time of analysis was 51 (range 1-192) months. Most of the patients (40/53) were treated with hydroxyurea either alone or in combination with aspirin, interferon or anagrelide for a median period of 55 (range 2-217) months. Results. The del20q12 was detected in 19 out of 84 samples (23%). The chromosomal aberration was revealed in 7/34 (21%) PV patients, in 9/38 (24%) ET patients and in 2/3 (67%) Unclassified CMPD. Sequential I-FISH studies were performed in six patients. In one PV patient, although the initial study was normal, the del20q12 was developed after a period of 21 months. The del20q12 was significantly associated with treatment failure (p=0.012) and inferior outcome of the disease. The five years survival without disease progression (myelofibrosis, secondary leukemia or myelodysplastic syndromes and death) was 62±15 months vs 89±5 months in patients with or without del20q12 respectively. Thus, patients with del20q12 were associated with significant increased odds of having disease progression (OR=3.1, 95% CI=1.1-8.6) and inferior outcome of the disease. Conclusion. Our experience, in concordance with other studies, showed that the del20q12 can be revealed by I-FISH in approximately 20% of CMPD patients. Poor prognosis and treatment failure were statistically associated with the del20q12. Larger and prospective series are needed to ascertain the possible clinical implications of del20q12 in CMPD patients.

0100

ABNORMAL EXPRESSION OF THE WAP FAMILY SLPI GENE IN PATIENTS WITH CHRONIC MYELOPROLIFERATIVE DISORDERS

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Azi Ospedaliere-Univeristaria Careggi, FLORENCE, Italy

Background. Abnormal expression of several protease inhibitors has been demonstrated to occur in granulocytes from patients with polycythemia vera (PV) using gene expression profiling (Pellagatti et al. Cancer Res, 2005). Among these, SLPI, a member of the WAP (whey-acidic-protein) family that has been involved in cell cycle, apoptosis regulation, endocytosis and expression of a lineage specific phenotype in several solid tumors was among mostly upregulated genes. Abnormalities of 20q11.13, where SLPI maps, have been reported in 10-25% of patients with idiopathic myelofibrosis (IM). AIMS The aim of the study was to evaluate whether abnormal expression of SLPI characterizes patients with MPD, and whether it might help in distinguishing between the different clinical entities. Methods. We studied 102 patients with MPD (28 PV, 58 ET, 21 IM) diagnosed according to WHO criteria, and 39 blood donors as healthy controls. Expression levels of SLPI and PRV-1 were determined by a quantitative TaqMan RT-PCR using granulocyte RNA and GAPDH as the housekeeping gene. In healthy donors the mean CT ratio for SLPI was 3.06±1.60 (range 1.92-4.67) and for PRV-1 3.81±0.92 (p=0.06) and to ET patients (4.98±1.85, p<0.0001); levels in ET patients were also significantly lower than in PV (p<0.005). By using the 4.67 cut-off, 66/102 patients had SLPI overexpression (65%), accounting for 96% (22/25), 43% (25/58) and 90% (19/21) of PV, ET and IM patients, respectively. The abnormal expression of SLPI correlated with the JAK2 mutational status: SLPI ratio was 3.16±1.62 in homozygotes, 4.35±1.78 in heterozygotes and 4.36±1.83 in wild-type patients; the difference between homozygotes and heterozygotes was statistically significant (p=0.02). There was no significant correlation between the overexpression of SLPI and that of PRV-1. To evaluate whether SLPI expression was modulated by cytokine exposure, as it has been shown for PRV1, we measured changes in SLPI mRNA levels after in vitro granulocyte activation with different cytokines such as G-CSF, IL8, IL3, IL11, IFN- and TNF-α. There were no significant modulation of SLPI unlike PRV1, that was induced by G-CSF exposure. SLPI plasma levels were measured by an ELISA assay; the mean plasma levels in MPD patients were significantly higher than in controls (4.8±1.9 ng/ml in MPD, 3.6±1.2 in healthy donors, p=0.06) and inferior outcome of the disease. Conclusion. In this study we have identified abnormal expression of SLPI gene as a novel molecular marker of MPD, that is also associated with raised plasma protein levels; there was also a disease-specific pattern of overexpression among IM, PV, and ET patients. The role of abnormal expression of SLPI in disease pathogenesis remains to be established.

0101

CONGENITAL ERYTHROCYTOSIS AND POLYCYTHEMIA VERA IN CHILDREN AND ADOLESCENTS - AN ONLINE DATABASE FOR REGISTRATION OF PATIENTS AND DATA COLLECTION

M.C. Bento,1 H. Cario,2 J. Vidan,1 A. Villegas,3 M.L. Ribeiro1

1‘Centro Hospitalar Coimbra, COIMBRA, Portugal; 2‘Childrens Hospital, ULM, Germany; 3‘UHM, Centro Hospitalar Coimbra, COIMBRA, Portugal; 4Grupo Español de Entropatología, MADERIA, Spain

Congenital erythrocytosis and Polycythemia Vera (PV) in children and adolescents represent rare and heterogeneous clinical entities. The current knowledge on the clinical presentation, laboratory investigation, as well as on the evolution and treatment of these disorders is sparse. Aim: In order to better characterize congenital primary and secondary erythrocytoses and PV in young patients, we developed an online computerized database for systematic registration of patients’ data. Material and Methods. Patients at any age with apparently congenital erythrocytosis and patients younger than 20 years with PV. Neonatal polglobulony will not be included. Selected patients with acquired secondary erythrocytosis can be followed as observational patients and may be included in the evaluation of particular aspects of the study. Diagnostic guidelines for apparently congenital erythrocytosis include the exclusion of potential underlying cardiac, pulmonary and renal disorders; the assess-
often be difficult and currently relies on clinical and biological criteria of Polycythemia Vera (PV) and Essential Thrombocytemia (ET) can mainly be secondary forms and result from several causes. The diagnosis presentation, on results of various diagnostic procedures and the clinical information. Reactive thrombocytosis (RT) was diagnosed in 6 out 11 T, a splenic infarction) and is currently treated with oral anticoagulant therapy. Background. Erythrocytosis (E) and thrombocytosis (T) in childhood are mainly secondary forms and result from several causes. The diagnosis of Polycythemia Vera (PV) and Essential Thrombocytemia (ET) can often be difficult and currently relies on clinical and biological criteria defined by WHO. Recently, an activating somatic point mutation of Jak2 has been described in the vast majority of patients with PV as well in subsets of patients with ET. Presence of this mutation is highly correlated with thrombosis and in 13 from 24 who do not develop thrombosis.

Methods. We conducted a retrospective study on all patients affected by E or T (including criteria: Hct> +2DS of the expected value or platelet count ≥ 1x10^6/mmc) referred to our Centre between 1st January 2000 and 31st December 2005. Results. Thirteen patients with E and eleven patients with T (M/F: 10/3 and 4/7; median age at diagnosis: 88 and 14 months, respectively) were investigated. 4/13 E resulted secondary to congenital heart disease, 1/13 secondary to persistent obstructive sleep apnea, 2/13 familiar forms and 6/13 primary erythrocytosis (PE). Of 8 PV was diagnosed as PV according to WHO criteria; he showed thrombotic complications (a cerebral ischemia and a splenic infarction) and is currently treated with oral anticoagulant therapy, low-dose aspirin, hydroxyurea and regularly undergoes to phlebotomy. Reactive thrombocytosis (RT) was diagnosed in 6 out 11 T, associated with bacterial or viral infections (mean duration = 4.4 months) while ET was diagnosed in 5 (mean duration = 45 months) according to WHO criteria (see clinical features of PE and ET in Table 1).

As far as the maximum platelet count is concerned no significant difference was found between ET and RT (mean = 1770±758 and 1510±591±10^3/mmc, respectively). On the contrary the duration of disease is significantly lower in RT (p<0.001). No thrombotic complications in T were documented; 3/6 RT and 5/5 ET treated with low-dose aspirin even if no prothrombotic factors were identified; 5/5 ET received also hydroxyurea, two of them received also anagrelide which was discontinued for important side effects. An isolated increase of PRV1 expression was observed in one PE and the presence of the missense mutation (V617F) in Jak2 gene associated with PRV1 over-expression was only found in the patient with PV. No molecular alterations were detected in TE patients. In all patients (n=22) was excluded. Cyto genetic analysis did not show abnormalities. Discussion. PV and TE are extremely rare diseases in childhood and therefore few paediatric data on incidence, clinical significance and management are reported in literature. Regarding T, the maximum level of platelet count does not allow to differentiate between RT and TE while the platelet count normalization in few months suggests a secondary form. Molecular markers, useful in distinguishing between MPD and secondary forms, are rare in our patients. Collaborative studies are necessary to better define clinical features, diagnostic approach and therapeutic strategies.

0103
PREDICTIVE VALUE OF ALTERATIONS OF COAGULATION AND THE JAK2 MUTATION ON THE RISK OF THROMBOSIS IN ESSENTIAL THROMBOCYTHEMIA
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Background. Essential thrombocytemia (ET) is a heterogeneous disorder in which thromboembolism remains the major cause of morbidity and mortality. Previous studies have shown high frequency of thrombophilia associated to alterations associated to hypercoagulability or thrombophilia and more recently in patients with the JAK2 V617F mutation.

Aims: First objective of this study was to study the prevalence of coagulation alterations associated with risk of thrombosis in patients with ET and the second aim was to analyze the incidence of JAK2 mutation in those patients and its relation with haematological parameters and risk of thrombosis.

Methods. We have studied 58 patients previously diagnosed of ET. Sex distribution was 25 males and 33 females. Age: ranged from 20 to 86 years (mean 59±15). Patients follow up was from six to 253 months (mean 89±54). In all patients, the presence of thrombophilia alterations was studied. The studies included: APTT, von Willebrand factor, Protein C, Protein S, S and Antithrombin III, PCAR, and presence of antiphospholipid antibodies. JAK2 mutation was analyzed in 32 patients. The significance of this mutation was investigated in relation with, haemoglobin, leukocytes and platelets levels, diagnosis of thrombosis, and with the diagnosis of thrombosis. Statistical analysis was performed with SPSS software. Results. To 1ers objective: 17 patients (29%) develop thrombosis, 7 before diagnosis and 10 during evolution. 59% of thrombosis were in patients older than 60 years, mean age of thrombosis was 61 years old. 50% of patients with thrombosis showed high expression of von Willebrand antigen factor; Results in both groups of patients were (Mean±SE: 159±51 and 128±48) respectively. VLeiden, P20210A, MTHFR mutation, low levels of protein C, S and Antithrombin III, PCAR, and presence of antiphospholipid antibodies did not appear as independent risk factors. However, 83% of thrombosis happened in patients with one or two alterations in the study of thrombophilia. To 2 objective: 1 JAK2 mutation was present in 56% of the ET patients. Patients with the mutation presented higher hematocrit values (44±5 vs 40±8; p=0.04), higher white blood cell counts (8951 vs 7304; p=0.05) and higher mean number of platelets (90800 and 685000 respectively; p=0.01). JAK2 Mutation was present in 5 from 8 patients with thrombosis and in 13 from 24 who do not develop thrombosis. JAK2 thrombosis risk in ET is increased in patients with alterations of coagulation, however none of these alterations shows independent predictive value. 2. Results from our serie confirm the presence of JAK2 mutation in more than 50% of ET patients and shows relation of this mutation with hematocrit value and higher white cell and platelets counts. 3. Relations of JAK2 and thrombosis cannot be established at the present time out of prospective studies.
Acute myeloid leukemia I

0104
MOLECULAR PROFILING USING ARRAY-CGH AND GENE EXPRESSION PROFILING REVEALS NEW CANDIDATE GENES IN AML WITH NORMAL KARYOTYPE

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Clonal chromosome abnormalities represent one of the most important prognostic factors in adult acute myeloid leukemia (AML), and cytogenetic data are used for risk-adapted treatment strategies. By conventional cytogenetic analysis, approximately 50% of patients lack clonal chromosome aberrations, and normal cytogenetics are associated with an intermediate clinical outcome. This clinically heterogeneous group seems to be in part characterized by molecular markers, such as MLL, FLT3, CE/BPA, and NPM1 mutations. In order to identify novel candidate regions of genomic imbalances, we applied comparative genomic hybridization to microarrays (array-CGH). Using this high-resolution genome-wide screening approach, we analyzed 49 normal karyotype AML cases characterized for the most common clinically relevant molecular markers (MLL-PTD n=15, FLT3-ITD n=7, FLT3-ITD/NPM1 n=4, MLL-PTD/FLT3-ITD n=3, CE/BPA n=12, CE/BPA/FLT3-ITD n=1; CE/PA+/NPM1 n=1; no molecular markers n=8) with a microarray platform consisting of 2799 different BAC or PAC clones. In addition to known copy number polymorphisms in 5q11, 7q21, 7q34, 14q32, and 15q15, we were able to detect copy number alterations (CNAs) in terms of gains in 9p, 11q, 13q and losses in 5p, 9p, 11q, 12p, 13q, and 16p. In a subset of cases we profiled global gene expression and the correlation of array-CGH findings with global gene expression profiles allowed the identification of candidate genes, e.g., FOXP1 and RYBP in 16p. In a subset of cases we profiled global gene expression and the correlation of high-resolution genomic profiling with global gene expression studies will help to disclose pathways underlying normal karyotypic AML, thereby leading to new insights of leukemogenesis.

0105
CO-EXPRESSION OF CD34, MDR1 AND BCRP INDICATES A CLINICALLY RESISTANT PHENOTYPE IN PATIENTS WITH ACUTE MYELOID LEUKEMIA OF OLDER AGE

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Clinical resistance to chemotherapy in acute myeloid leukemia (AML) is often associated with the expression of the multidrug resistance (MDR) proteins P-glycoprotein (P-gp), encoded by the MDRI/ABCB1 gene, multidrug resistant-related protein (MRPI/ABCG2), the lung resistance-related protein (LRRP), or major vault protein (MVP), and the breast cancer resistance protein (BCRP/ABC2). The clinical value of MDRI, MRPI, LRP/MVP and BCRP mRNA expression was prospectively studied in 154 newly diagnosed AML patients aged ≥ 60 years, who were treated in a randomized clinical trial. Expression of MDRI and BCRP showed a negative while MRPI and LRP showed a positive correlation with high white blood cell count and LAIP-positive cells at follow-up assessment. Results. The median LD amounted to 2.18 (range, -0.03 to 4.17) at CP2, 2.49 (0.11 to 4.17) at CP3, 2.58 (-0.28 to 4.28) at CP4, and 2.87 (0.46 to 4.02) at CP5. A higher LD (continuous variable) was associated with worse event-free survival (EFS) and overall survival (OS), using univariate analysis. Although CD34 expression overruled all other prognostic factors, co-expression of MDRI and BCRP identified a clinically resistant subgroup of elderly AML patients with a low CR rate and poor EFS and OS (p-values respectively: 0.01, 0.01 and 0.05).

0106
REPLICATIVE SENESCENCE INDUCTION IN GOOD-RISK ACUTE MYELOID LEUKEMIA

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Immortal cell growth is considered the hallmark of tumor cells. In contrast, normal cells have a limited proliferative capacity of 40-60 cell divisions, also known as the Hayflick limit. The limited proliferative capacity of normal cells relates to gradual shortening of telomeric DNA as a consequence of the end-replication problem. Upon critical shortening of telomeric DNA, cells enter a non-replicative but viable state referred to as replicative senescence. These replicative senescent cells stain blue in a β-Galactosidase assay. Human fibroblast models have shown that escape from senescence results from loss of p53 and Rb function. Escape is associated with activation of a telomere maintenance mechanism, which contains telomerase gene activation and telomere elongation mediated by telomeric DNA. High levels of telomerase, as observed in germ cells and most tumor cells, allow for immortal cell growth. Recently, we demonstrated relatively low levels of telomerase in AML patients with t(8;21) or inv(16) (Swiggers et al., GCC 2006). Interestingly, levels of telomerase in AML cases characterized for the most common clinically relevant molecular marker, MLL-PTD, showed relatively low levels of telomerase in normal bone marrow progenitor cells. We hypothesized that these AML cells, where telomerase is not reactivated to high levels, may not have inactivated the senescence pathways that limit the proliferative capacity of normal cells. This hypothesis was addressed by studying AML patient samples with t(8;21), t(15;17) or inv(16) in vitro (long-term cell cultures in presence of growth factors) and in vivo (following transplantation in NOD-SCID mice and in patients at time of relapse) for cells with all characteristics of replicative senescence, i.e., viable, non-proliferating, blue-coloring in β-Gal assay, and critical short telomeres. AML cells with all characteristics of replicative senescence were clearly observed in AML samples with either t(8;21), t(15;17) or inv(16). Gradual telomere shortening was observed in these AML cell lines under long-term culture, in vivo following transplantation in NOD-SCID mice and in vivo in patients at relapse, indicating that these AML cells do not have an adequate telomere maintenance mechanism. We included in the study a control group of AML that is characterized by telomerase reactivation in high levels in a cubic karyotypic group, n=8. Cells with characteristics of replicative senescence were not induced in vitro or in vivo in any of the samples of this AML control group. We conclude that AML cells with t(8;21), t(15;17) or inv(16) are characterized by intact pathways that induce replicative senescence. Intact pathways that limit proliferative lifespan may be critical to the high cure rates following chemotherapy treatment of patients with good-risk AML.

0107
PROGNOSTIC IMPACT OF FLOW CYTOMETRICALLY DETERMINED MINIMAL RESIDUAL DISEASE IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background. Adaptation of treatment in acute myeloid leukemia (AML) is based on individual risk profiles. Significant prognostic information may be provided by the levels of minimal residual disease (MRD). Aims. To assess the prognostic impact of flow cytometrically quantified MRD levels in AML in a multivariate analysis. Methods. We applied multiparameter flow cytometry (triple staining) to highly sensitively quantify MRD in patients with AML. A total of 586 patients receiving standardized intensive antileukemic treatment was analyzed at different checkpoints: CP1 (up to day 28, n=156), CP2 (day 28-60, n=122), CP3 (day 61-120, n=195), CP4 (day 121-365, n=172), and CP5 (after day 365, n=73). Leukemic cells were identified by their individually defined leukemia-associated aberrant immunophenotypes (LAIPs). MRD levels were calculated as the logarithmic difference (LD) between LAIP-positive cells and LAIP-negative cells at follow-up assessment. Results. The median LD amounted to 2.18 (range, -0.14 to 4.20) at CP1, 2.31 (-0.03 to 4.17) at CP2, 2.49 (0.11 to 4.17) at CP3, 2.58 (-0.28 to 4.28) at CP4, and 2.87 (0.46 to 4.02) at CP5. A higher LD (continuous variable) was
related to a better event-free survival (EFS; CP1, p=0.0002; CP2, p=0.00001; CP3, p=0.0002; CP4, p<0.00001; CP5, p=0.00007) and to a better overall survival (OS; CP1, p=0.004; CP2, p=0.001; CP3, p=0.021; CP4, p=0.00006). Other parameters related to EFS and OS were age and cytogenetics in the present series. The prognostic impact of MRD levels on outcome was independent of cytogenetics and age for EFS (CP2 to CP5) and OS (CP2 and CP4). MRD was the most important prognostic parameter at CP4 and CP5. Separation of patients into two groups, respectively, by the median LD resulted in significant differences in EFS at all CPs and in OS at CP1 to CP4. The largest difference was observed at CP4: median EFS, 57.1 vs. 15.7 months, p<0.00001; 3-year-OS, 95% vs. 65%, p=0.0005. Summary. A highly powerful and independent prognostic parameter is provided by the MRD levels determined by multiparameter flow cytometry which is applicable to the total of an AML population. It should be evaluated as a stratification parameter in clinical trials.

0108
PROGNOSTIC RELEVANCE OF FLT3-TKD MUTATIONS IN AML: THE COMBINATION MATTERS–AN ANALYSIS OF 3082 PATIENTS
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Background. FLT3-TKD mutations are located in the activation loop domain of the FLT3 gene and mostly represent point mutations in codon D835 or deletions of codon 836. They induce constitutive activation of the receptor tyrosine kinase and are supposed to represent gain-of-function mutations. These mutations have been described in AML previously, but in contrast to FLT3-LM/ITDs the distribution within AML subtypes and an impact on prognosis has not been reported, so far. Methods. We screened 3082 newly diagnosed patients with AML for this mutation by a LightCycler based melting curve analysis. Positive cases were further characterized by sequencing. Results. FLT3-TKD mutations were found in 147/3082 (4.8%) of all patients. Most patients revealed amino acid exchanges from D to Y at position 835 (68/137; 46.6%). Monoallelic mutations occurred in 118 cases (96.7%), whereas biallelic mutations accounted for 4 of 122 mutated cases (2.9%). In total, FLT3-TKD mutations occurred in nearly each cytogenetic subgroup. Similar to FLT3-LM/ITDs there was a significant correlation of FLT3-TKD mutations with t(15;17)/PML-RARA (10/130; 7.7%), whereas the incidence in normal karyotype was not significantly higher as compared to others. In addition, FLT3-TKD mutations were significantly underrepresented in complex aberrant karyotype (4/450; 0.9%), in 5q-/5 (4/11; 0%), and in 7q-7 (7/59 (1.2%)), i.e. in the prognostically unfavorable subtypes. The incidence was low in t(8;21)/AML1-ETO (2/68; 2.3%). With regard to morphology, FLT3-TKD mutations were overrepresented in the FAB subtype AML M3v (6/51; 11.8%) and in the FAB subtypes M1 (42/491, 6.6%), M4 (59/484 (12.1%)), and M5b (15/114 (13.2%))). In contrast to FLT3-LM, FLT3-TKD mutations were more frequently associated with higher peripheral leukocyte counts, FLT3-TKD did not correlate with this parameter. A correlation of FLT3-TKD with other molecular mutations showed the highest incidence of FLT3-TKD mutations in cases with NPM1 (23/262; 8.8%), CEP11 (6/76; 7.9%), and NRAS mutations (6/78; 7.7%). FLT3-TKD in combination with FLT3-LM (17/594 patients; 2.9%) and KITD816 (1/44; 2.3%) was rare. In contrast to FLT3-LM which is known as strong negative prognostic parameter, overall survival (OS) and event free survival (EFS) were influenced neither in the total cohort (57 FLT3-TKD and 1623 FLT3-WT) nor in the normal karyotype group (97 FLT3-TKD and 764 FLT3-WT). However, in the subgroup with t(15;17)/PML-RARA EFS was unfavourably influenced by FLT3-TKD mutations. With regard to other molecular mutations there was an additional unfavourable impact for FLT3-LM/TKD double mutated and MLL-PTD/TKD double mutated cases. In contrast, there was an additional favourable impact on EFS in NPM1 and in CEPBA. Conclusion. Thus, the FLT3-TKD mutations seem to have an unfavourable enhancement of unfavourable and a favourable intensification of prognostically favourable molecular mutations.

0109
EXPRESSION OF TUMOR-ASSOCIATED ANTIGENS IN ACUTE MYELOID LEUKEMIA AND THEIR CORRELATION WITH SURVIVAL
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Background. The expression of tumor-associated antigens (TAAs) might play a critical role in the control of minimal residual disease (MRD) in acute myeloid leukemia (AML). Aims. Here, we investigated whether TAAs were associated with clinical outcome in AML. Methods. A DNA-microarray analysis of 116 AML samples as well as ELISPOT, FACS and chromosome release assays were performed to assess TAA-specific T cell responses in these patients. Results. A significant correlation of high mRNA expression of G250/CA9 with a longer overall survival (p=0.022), a trend for better outcome in patients with high expression levels of PRAME (p=0.105), and a hint for RHAMM/HMMR. In contrast, for other TAAs like WTI, TERT, PRTN3, BCL2, and LAMR1 we found no correlation with clinical outcome. Interestingly, the co-expression of RHAMM/HMMR, PRAME and G250/CA9 provided a favorable prognostic effect (p=0.005). We also observed specific T cell responses at high frequency for these antigens. Positive immune reactions were detected in 8/17 (47%) AML patients for RHAMM/HMMR-derived, in 7/10 (70%) for PRAME-derived, and in 6/10 (60%) for newly characterized G250/CA9-G2-derived peptides. Furthermore, we could demonstrate specific lysis of T2 cells presenting these epitope peptides. Conclusion. The expression of the TAAs RHAMM/HMMR, PRAME and G250/CA9 can induce strong anti-leukemic immune responses possibly enabling the control of MRD in AML patients. Thus, these TAAs represent interesting targets for polyvalent immunotherapeutic approaches.

0110
PROGNOSIS OF ACUTE MYELOID LEUKEMIA PATIENTS < 60 YEARS WITH TRISOMY 8 AS A SOLE ABERRATION: POOLED DATA ANALYSIS OF THE GERMAN ACUTE MYELOID LEUKEMIA INTERGROUP
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Background. Trisomy 8 (+8) occurs in about 8-13% of patients with acute myeloid leukemia (AML). However, so far no prognostic impact and best consolidation strategy of this recurrent aberration is unclear. Thus, additional prognostic factors are needed to further classify those patients and to deliver appropriate treatment. Methods. Pooled data analysis was performed on 198 adult patients (median age 49 (17-60) years) with +8 treated between 1995 and 2002 in eight prospective German AML treatment trials. Patients with t(8;21), inv(16) and an additional +8 were not included in the study. Clinical, diagnostic and laboratory data were reviewed for consistency and completeness before analysis by a central coordination center Results. Ninety-two (46%) patients had +8 as a sole aberration, 59 (20%) had one additional secondary aberration and 67 (34%) had +8 within complex karyotypes with at least three independent abnormalities. Patients with +8 as a sole aberration had a 3-year overall survival (OS) and relapse-free survival (RFS) of 27% (95%-CI 18%-36%) and 31% (95%-CI 18%-43%), respectively. Multivariate analysis including standard clinical and laboratory data, as well as percentage of +8 positive metaphases and FLT3 status revealed extramedullary disease at diagnosis as a significant prognostic variable for worse survival (HR 2.56 (95%-CI 1.38-4.75); p=0.06), whereas post-remission therapy (i.e. high-dose cytarabine vs. autologous vs. allogeneic stem cell transplantation) did not influence survival. Conclusion. AML patients with +8 as a sole aberration can be stratified by extramedullary disease at diagnosis into two prognostic groups. However, alternative treatment approaches are needed to achieve more durable remissions in this AML entity in the future.
0111
PROGNOSTIC IMPACT OF THE NPM1/FLT3 ITD MUTATION STATUS IN ELDERLY PATIENTS >60 YEARS OF AGE WITH NORMAL KARYOTYPE AML: RESULTS OF AMLSG TREATMENT TRIAL AML HD98B
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Background. NPM1 mutations have been identified in approximately 50% of patients with normal karyotype acute myeloid leukemia (AML) and thus represent the most frequent genetic alteration in this subset of patients. In three recent studies mainly involving younger adults, statistical analysis revealed a significant interaction between NPM1 mutations and FLT3 internal tandem duplications (ITD). Only patients with NPM1 mutation in the absence of FLT3 ITD had a significantly better relapse free survival (RFS) and overall survival (OS).

Aims. To evaluate the prognostic impact of the combined NPM1/FLT3 ITD mutation status on response to therapy, RFS and OS in elderly AML (≥60 years) with normal karyotype.

Methods. So far, sequencing of NPM1 exon 12 mutations was performed in diagnostic samples from 84 patients entered into the AML HD98B treatment trial of the AML Study Group (AMLSG). Treatment included randomized induction therapy consisting of 2 courses of ICE (idarubicin, cytarabine, etoposide) with or without all-trans retinoic acid (ATRA) followed by first consolidation therapy with a course of HAM (cytarabine, mitoxantrone) with or without ATRA. For further postremission therapy, patients were randomized to one cycle intensive second consolidation therapy (idarubicin, etoposide) or 12 monthly courses of outpatient maintenance therapy (idarubicin and etoposide per os). Results. NPM1 mutations were identified in 43% of the leukemias. In analogy to the studies performed in younger AML patients, statistical analysis revealed a significant interaction of NPM1 and FLT3 ITD mutations. Only the NPM1+/FLT3 ITD- genotype predicted for high response to induction therapy and better survival probabilities. Complete remission rate (CR) of this subgroup was significantly better than that in the other three subgroups (NPM1-/FLT3 ITD-; NPM1+/FLT3 ITD+; NPM1-/FLT3 ITD+). Treatment failure in the three latter groups was due to a higher degree of refractory leukemias (55% vs. 20.5%). The higher response to therapy translated into significant better RFS (p<0.001) and OS (p=0.01) probabilities in the NPM1+/FLT3 ITD- compared to the three other genotypes. Conclusions. The NPM1+/FLT3 ITD- genotype defines a distinct subset of elderly AML patients with a favorable outcome. These data are most relevant when aiming at selecting those elderly patients who may benefit from intensive therapy and those who will not.

0112
PROGNOSTIC DETERMINANTS IN ADULT AML PATIENTS WITH INTERMEDIATE RISK KARYOTYPE
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Background. According to the prognostic classifications of karyotypic abnormalities of AML (MRC and SWOG), the intermediate group includes patients either lacking good and poor karyotype or with normal karyotype. Therefore, it represents, by definition, a miscellaneous group for which the evaluation of the better treatment strategy is difficult due to its heterogeneity. Moreover, patients belonging to this intermediate group account for the large majority of AML cases enrolled into clinical trials.

Aims. The aim of our study was to analyze the factors specifically affecting the outcome of patients bearing intermediate risk karyotypic abnormalities in a group of 94 AML cases entered into the EORTC/GIMEMA protocols AML10/AML12 (age <61yrs) or AML13/AML15 (age ≥61yrs), consisting in intensive induction and consolidation therapy. Methods. The clinico-biological variables evaluated in our model included age, FAB, WBC count, MDR1 phenotype, FLT3 mutations and level of post-consolidation bone marrow residual leukemic cells (BMRLC), assessed by multiparametric flow-cytometry (MPFC). By applying the maximally selected log-rank statistics, the threshold discriminating MDR negative from positive cases was set at 3.5x10^4 BMRLC, a level that allowed the identification of distinct subgroups of MDR- and MDR+ patients, both at post-Ind and post-Cons
time-points. Results. Patients with <3.5x10^4 BMRLC at the end of consolidation therapy were considered MDR- and showed a better outcome, patients whose level of MDR were ≥3.5x10^4 at the end of consolidation were considered MDR+ and showed a poor prognosis. Using the MRC classification, 14/94 patients (15%) had a good-risk cytogenetics, 74/94 (79%) an intermediate-risk and 6/94 (6%) a poor-risk. When we restricted the analysis to cases with intermediate-risk karyotype we found that: 1) patients in the MDR- and MDR+ group differed significantly in terms of relapse free survival (RFS), overall survival (OS) and relapse rate (p<0.001, 0.006 and <0.001, respectively); 2) MDR- patients had an outcome slightly better than those bearing risk karyotype; 3) MDR+ patients showed a dismal outcome comparable to poor-risk cytogenetic patients. Conclusions. These results suggest that the inclusion of BMRLC assessment of MDR in patients with intermediate risk karyotype may be particularly useful in discriminating subgroups with different outcomes, in a group of AML where karyotype does not represent a clear prognosticator, allowing clinicians to design risk-based therapeutic programs.
0114 TRISOMY 8 AS SOLE ANOMALY OR WITH OTHER CLINICAL ABERRATIONS IN ACUTE MYELOID LEUKEMIA: IMPACT OF CLINICAL PRESENTATION AND TREATMENT OUTCOME
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Background. Trisomy 8 is the most frequent numerical aberration in acute myeloid leukemia (AML). It occurs either as the sole anomaly or together with other clonal chromosome aberrations. Only few data are available regarding their prognostic significance. Aims. In order to investigate whether accompanying chromosome anomalies influence the clinical outcome in patients with trisomy 8, we assessed clinical and biological characteristics, and response to therapy, of an unselected group of patients with previously untreated AML, presenting with trisomy 8 either alone or with other clonal aberrations. Methods. One hundred and fifty-four cases (median age: 65 years) were diagnosed in our institution between 1981 and 2005 including 47 patients (31%) with trisomy 8 as the sole aberration, 107 patients (69%) with trisomy 8 associated with other cytogenetic abnormalities (12% with favorable risk, 54% with intermediate risk, and 40% with unfavorable risk cytogenetics). Results. Twenty-eight patients only received symptomatic therapy or died before any chemotherapy could be given. All other patients received induction treatment according to different protocols used during the period of study. Overall complete remission (CR) proportion was 48% (95% confidence interval (CI): 40 - 56%). Sixty-six patients achieved CR after one course of chemotherapy and 8 patients after salvage therapy. Median disease-free survival (DFS) of the entire cohort was 7.8 months (95% CI: 6.5-9.9 months) and median overall survival (OS) was 8.3 months (95% CI: 5.2 - 9.8 months). In multivariate analysis, age more than 60 years and trisomy 8 associated with unfavorable chromosomal aberrations were of poor prognostic value for CR achievement. Age more than 60 years and antecedents of dysmyelopoiesis were of poor prognostic value for DFS and OS. Patients with trisomy 8 alone did not show a significant difference in terms of outcome as compared with those in whom trisomy 8 was associated to intermediate risk chromosomal aberrations. Patients with trisomy 8 in addition to favorable chromosome aberrations maintained a good clinical outcome, while those with trisomy 8 in addition to unfavorable karyotypes showed the worst prognosis. Conclusions. Trisomy 8 as a whole has poor survival, which is largely attributable to worsened outcomes among patients whose trisomy 8 was associated with unfavorable cytogenetic abnormalities. A particular poor outcome was observed in patients presenting trisomy 8 with antecedents of myelodysplasia.

0115 ANALYSIS OF 1458 ACUTE MYELOID LEUKEMIA FROM THE ALERT PROJECT (ACUTE LEUKEMIA CLINICAL REGISTER) IN THE CZECH REPUBLIC IN 1996-2006

Project ALERT was initiated 9 years ago as population registry for acute leukemias, based on the cooperation of large haematological centres in the Czech Republic. Although the primary aims were clinical than epidemiological, the growing database tends to become representative for the Czech population, at least in the category of intensive and standard dose consolidation chemotherapy; pts who are treated with current regimens could receive a consolidation course of 400 mg/m² as a single 80-60 min. IV infusion. Second induction was allowed for patients who showed improvement. Patients who achieved CR or CRp could receive a consolidation course of 400 mg/m².

Figure 1. % Probability of Overall Survival.

Results. 105 pts were treated (median age 72, range 60-84), of whom 45 (43%) had de novo AML, 45 (43%) had secondary AML and 15 (14%) had high risk MDS. Twenty-eight pts achieved a CR and 8 pts a CRp for...
an overall response rate of 31%. Response in 45 de novo AML, 45 second or subsequent remission, and 32% in high-risk MDS with a trend towards improved survival. Response by cytogenetics was 42% in 57 intermediate pts and 22% in 41 unfavorable pts. The CR rates achieved with Clodaretine are consistent despite increasing age and declining performance status. Severe drug-related non-hematologic toxicity was rare. Nineteen (18%) pts died within 30 days of receiving Clodaretine. As demonstrated in the figure below, higher troxacitabine Css concentrations were achieved for all treated pts (N=103) was 12% and 28% for pts with CR. Patients with de novo AML who achieved CR had a median survival of 5 months and a 1-year survival of 52% (N=22). Conclusion: Clodaretine, is well tolerated and has significant activity in an elderly patient population with AML or MDS. The encouraging activity in patients with de novo AML warrants further evaluation.

**0117 DOOSING OF TROXATYL (TROXACITABINE, SXG-145) BASED ON RENAL FUNCTION**

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**Background.** Troxatyl™ (troxacitabine, SXG-145) is a novel L-configuration nucleoside analog with unique mechanistic and cytotoxic properties. Troxatyl is clinically active in patients with relapsed or refractory acute myelogenous leukemia (AML), including those who have failed bone marrow transplantation. Troxatyl has also demonstrated clinical activity against chronic myeloid leukemia, myelodysplastic syndromes, renal cell carcinoma, and pancreatic cancer. An international (European and North American) multi-center Phase 2/3 clinical trial is currently underway to evaluate the safety and efficacy of troxatyl continuous IV infusion treatment in second salvage AML (SPD758-216; www.clinicaltrials.gov). The major route of elimination of troxatyl is renal excretion as unchanged drug (~70%) and there is no degradable protein binding. These results suggest that renal function may play a significant role in determining the troxatyl blood concentration in an individual patient. Aims. The aims of this study were to determine the influence of renal function on troxatyl steady-state plasma concentrations (Css), and to determine if a correlation exists between troxatyl Css and clinical response. Methods. Pharmacokinetic and toxicity data from multiple AML clinical trials (enrolling >200 patients) are being analyzed to (1) Determine the relationship between troxatyl Css values and estimated creatinine clearance; (2) Identify the minimum troxatyl Css required to achieve a clinical response (CR or CRp); (3) Define an upper limit of troxatyl Css for adverse risk; and (4) Develop a dosing nomogram or equation to prospectively adjust troxatyl dosing, based on patient renal function, which will allow avoidance of excessive toxicity while still achieving therapeutic blood levels. Results. Initial results indicate that in order to induce remission in relapsed or refractory AML patients, troxatyl Css values must ≥ 80 ng/mL. High troxatyl Css values may correlate with increased toxicity, although an upper limit for adverse risk has not yet been defined. For patients with normal to mildly impaired renal function (creatinine clearance > 45 but < 125 mL/min), a Calvert style formula, based on estimated creatinine clearance, has been developed to define the minimum dose required to achieve the target troxatyl Css of > 80 ng/mL. Both linear and non-linear nomogram models are being developed to adjust troxatyl dosage for patients with moderate impairment of renal function (creatinine clearance < 45 mL/min) or for patients with higher glomerular filtration rates (creatinine clearance > 125 mL/min). Summary. A firm relationship exists between renal function and troxatyl Css values, and between troxatyl Css values and clinical response in patients with relapsed or refractory AML. These results indicate that developing a dosing strategy, based on patient renal function, may be warranted to obtain optimal troxatyl Css values with minimal toxicity.

**0118 CYTOGENETIC RESPONSES IN OLDER PATIENTS WITH AML AND COMPLEX KARYOTYPE TREATED WITH LOW-DOSE 5-AZA-2-DEOXYCYTIDINE (DECITABINE)**

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**Background.** The demethylating agent Decitabine (DAC) induces hematologic and cytogenetic remissions in older patients (pts) with MDS and AML (Ruter et al Int J Hematol. 2004; 80: 128-35). Cytogenetic normalization was more frequently seen in pts with poor-risk cytogenetics (mostly complex karyotype) compared to intermediate-risk (Rüter et al Br J Haematol 2001; 114: 349). Aim. Prospective analysis of induction of cytogenetic and hematologic responses in AML pts aged ≥ 60 (median age 75) treated with DAC (15mg/m2 total dose) every 6 weeks for 2 courses. Results. A total of 121 pts were evaluable for cytogenetic remissions. 13 of the 21 pts (62%) had a complete cytogenetic normalization, all with a complex karyotype at diagnosis (9 with complex karyotype). 3/13 had chondromal abnormalities prior to treatment. Cytogenetic subgroups were: good risk: 0; intermediate risk: 21 pts (12 with normal karyotype); poor risk: 14 pts. Pts received a median of 2 DAC courses (range 1-4) and 10 pts a median of 2 maintenance courses (range, 1-9). 21/35 pts received ≥ 2 courses DAC (17 also ATRA) and were evaluable for cytogenetic remissions. 13 of the 21 pts (62%) had chondromal abnormalities at diagnosis (9 with complex karyotype). 3/13 had a complete cytogenetic normalization, all with a complex karyotype at time of diagnosis (aberrations of chromosome 5, 3, and chromosome 7 in 2). The cytogenetic response occurred after a median of 3.5 courses. Overall hematologic response in pts achieving a complete remission by intent-to-treat (ITT) was 45% (17/39) with 6 CR (15%), 4 PR (10%) and 7 antileukemic effect (ALE) (18%). Conclusions. Low-dose DAC is active, by ITT, in inducing hematologic response in 45% and cytogenetic normalization in 13% of older AML pts (according to FAB), all 3 with complex karyotype. In MDS pts cytogenetic normalization (after a median of 4 courses) had been seen in 7% (31%). ATRA pts received a median of 2 courses which could explain at least in part the lower cytogenetic response rate.
showed a weaker gain-of-function phenotype in terms of proliferation, anti-apoptosis, activation of FLT3 and the downstream target STAT5. As the FLT3-JM-PM cluster at a core interaction site of the JM domain with the remainder of the molecule we hypothesized that the oncogenic potential results from the perturbation of the autoinhibitory mechanism. Mapping of the FLT3-JM-PM on the crystal structure of FLT3 (figure) could show that these mutations probably reduce the stability of the autoinhibitory JM domain. In comparison to FLT3-ITD mutations, that increase the length of JM, the FLT3-JM-PM induce a considerably weaker perturbation and this might provide a structural basis for the weaker (FLT3-JM-PM) versus stronger (FLT3-ITD) transforming capacity of these mutations. Summary. A third class of activating FLT3 mutations exists in 2% of AML patients, point mutations in the structurally important JM domain (FLT3-JM-PM). Patients carrying FLT3-JM-PM might benefit from the treatment with selective FLT3 inhibitors. FLT3-JM-PM provide a remarkable example how mutations disturbing the autoinhibitory JM domain of class III RTK can contribute to the pathogenesis of cancer.

**0120**

ORIGINAL DATA BASED META-ANALYSIS ON PATIENTS >60 YEARS OF AGE WITH CORE BINDING FACTOR ACUTE MYELOID LEUKAEMIA RESULTS OF THE GERMAN AML-INTERGROUP


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Background. Acute myeloid leukaemias exhibiting t(8;21) or inv(16) karyotypes are referred to as core binding factor (CBF) acute myeloid leukaemia (AML). In younger patients with CBF-AML relapse-free survival (RFS) was markedly improved following dose-intensified cytarabine for consolidation therapy. However, in older patients (>60 years) the clinical course is characterized by significantly inferior outcome and the value of dose-intensified cytarabine remains unclear. Aims. To review the clinical course of CBF-AML in a large cohort of elderly patients and to define the relative value of different treatment strategies. Methods. We performed a meta-analysis on 65 patients with t(8;21) and on 51 patients with inv(16) from 4 German leukaemia study groups. The patients were treated in 2 different prospective multicenter treatment trials between 1995 and 2004 (DSIL n=36, AMLSG n=26, AMLCG n=34, OSHO n=20). Induction therapy consisted of standard dose cytarabine (ARAC) combined with etoposide/idarubicin or mitoxantrone, or dose-intensified cytarabine in combination with idarubicin or mitoxantrone; postremission therapy consisted of intensive chemotherapy followed by maintenance therapy in two trials. Results. The median age was 66 years (range 61-85) and median follow up time was 55 months. Response to induction therapy for t(8;21) and inv(16) was as follows: complete remission (CR) 72.5% and 86%, refractory disease (RD) 12% and 10%, early/hypoplastic deaths 15.5% and 4%, respectively. RFS and overall survival (OS) after 4 years were for t(8;21)-AML 20% (95%-CI 11-33%) and 20% (95%-CI 11-35%) and for inv(16)-AML 35% (95%-CI 23-54%) and 33% (95%-CI 22-51%), respectively. To evaluate the impact of dosage of cytarabine on outcome patients were categorized into the HiDAC-group if they received at least one cycle of high-dose cytarabine with a cumulative dosage of 26g/m2 (n=35) or otherwise into the STANDARD-dose cytarabine group (n=34). RFS was significantly (p=0.002) better in the HiDAC-group (44%, 95%-CI 30-65%) compared to the STANDARD-group (18%, 95%-CI 7-35%). Conclusion. Elderly patients with CBF-AML seem to benefit from dose-intensification of cytarabine above or equal to 6g/m2 in at least one treatment cycle.

**0121**

THE KINETICS OF REDUCTION OF MINIMAL RESIDUAL DISEASE IMPACTS ON DURATION OF RESPONSE AND SURVIVAL OF PATIENTS WITH ACUTE MYELOID LEUKAEMIA


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Background. We assessed by multiparametric flow-cytometry the levels of minimal residual disease (MRD) in 100 adult patients with acute myelogenous leukemia (AML) achieving complete remission after intensive chemotherapy. Aims. The aim of the present study was to determine the optimal threshold level, in terms of residual leukemic cells (RLC), and the time-point of choice, i.e. post-induction (post-Ind) or post-consolidation (post-Cons), capable to better predict outcome of AML patients. Methods. By applying the maximally selected log-rank statistics, the threshold discriminating MRD negative from positive cases was set at 3.5×10³ RLC, a level that allowed the identification of distinct subgroups of MRD+ and MRD+ patients, both at post-Ind and post-Cons time-points. Results. Post-Cons MRD+ patients had a superior outcome in terms of relapse rate, OS and RFS (p<0.001, for all comparisons), regardless of the MRD status after induction. In particular, patients entering MRD negativity only after consolidation showed the same outcome as those achieving early negativity after induction. Multivariate analysis including karyotype, age, MDRI phenotype, post induction and post consolidation MRD levels indicated that the post-consolidation MRD status was an independent factor affecting the outcome of AML patients. Conclusions. 1) the threshold of 3.5×10³ is valid in discriminating risk categories in adult AML; 2) MRD assessment at post-consolidation time-point is critical to predict disease outcome.

**0122**

CLOFARABINE AS FIRST-LINE TREATMENT OF ELDERLY (=65 YRS) AML PATIENTS WITH AN UNFAVOURABLE CYTOGENETIC PROFILE WHO ARE UNSUITABLE FOR STANDARD TREATMENT

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Background. BIOV-121 is a phase II non-randomised trial of clofarabine, a next generation purine nucleoside analogue, in older patients (≥65 yrs) with previously untreated acute myeloid leukaemia (AML) who are unsuitable for standard (3+7) chemotherapy. Aims. The primary endpoint of study BIOV-121 is to determine the overall response rate (ORR) of clofarabine in this elderly (≥65 yrs) AML population who are unsuitable for standard treatment. ORR is defined as the sum of the number of patients who achieve a complete response (CR), a complete response with incomplete peripheral count recovery (CRi) and a partial response (PR) according to the international working guidelines. Secondary endpoints include duration of remission, time to progression, and the safety and tolerability of clofarabine in this patient population. Methods. 66 patients aged ≥65 yrs with untreated AML defined by the WHO classification were enrolled in BIOV-121. All patients were considered unsuitable for standard treatment based primarily on age (≥65 yrs) and/or performance status. All patients received clofarabine 30mg/m² for 5 days repeated every 28 days (1 course). A preliminary analysis was conducted for patients with an unfavourable cytogenetic profile (16/68), for whom data was available, and for patients ≥70 years of age (36/66). Unfavourable cytogenetic profile was defined as the presence of a com-
Acute myeloid leukemia II

0124 IMMUNOPHENOTYPIC PATTERN OF HIGH RISK KARYOTYPE ACUTE MYELOID LEUKEMIA IS CHARACTERIZED BY EXPRESSION OF CD34 AND LACK OF MPO

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Background. Cyto genetic is nowadays the most remarkable prognostic factor in acute myeloid leukemia (AML), and immunophenotypic analysis may help to identify some of the most frequent cytogenic abnormalities in this disease. Aim. To identify immunophenotypic pattern amin in AML with adverse cytogenetics. Methods. Samples from a total number of 185 de novo AML patients (median age: 54 years, range 10-91; 56% male and 44% female) were immunophenotypically analyzed by multiparametric flow cytometry, using a large panel of 25 monoclonal antibodies. A cut-off point of 20% of the total blast cell population was used to define a marker as positive or negative. Chromosome abnormalities and bone marrow cellularity were performed using standard FISH. Results. Among the AML cases classified as high risk karyotype, 32/59 (53%) showed a significant immunophenotypic pattern in blast cells characterized by the CD34 expression and lack of expression of cytoplasmatic myeloperoxidase. In contrast, this pattern was observed in 4/61 (9%) and 10/51 (22%) of good and intermediate cytogenetic risk group respectively (p=0.001). Moreover, when patients with chromosome 3 abnormalities were considered as an isolated group (n=9), this pattern was present in 89% of cases, while AML with -5/-7q (n=10) and -7q (n=19) showed this immunophenotypic pattern in 30% and 53% of cases, respectively. By contrast, AML with good risk karyotypes (n=61) only showed this immunophenotypic pattern in 3/21 (14%) of AMLs with t(8;21), 1/21 (5%) of inv(16) and no cases with t(15;17). Therefore, once the CD34+/MPO- immunophenotypic pattern is identified, the relative risk of bearing a cytogenetic abnormality belonging to the adverse cytogenetic group in de novo AML patients is 3.2 times when compared to the immunophenotypic profiles. Conclusions. The immunophenotypic pattern characterized by the expression of CD34 and lack of expression of MPO (CD34+MPO-) is associated with adverse prognosis karyotype. Additional cytogenetic studies (e.g. FISH) should be performed in cases with normal or unsuccessful immunogenetics analysis in order to identify possible overlooked abnormalities.

0125 ABNORMALITIES IN P53 AND P14ARF IN DE NOVO AML PREDICTS A VERY SHORT OVER-ALL SURVIVAL AND IN VITRO DRUG-RESISTANCE.

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The purpose of this study was to correlate the karyotype of myeloblast to long-term overall survival and in vitro cytotoxicity of conventional antileukemic drugs in patients with de novo AML. Abnormalities in chromosome 17, which are associated with p53 mutations, and 9p, the locus that encodes among others p14ARF which binds and inactivates the HDM-2, which in turn targets p53 for degradation. Thus deletions in 9p21 resulting in inhibitory effects on p53 protein were focused on. Methods. Blast cells were isolated from 521 patients diagnosed with de novo AML during the last 20 years at our clinic. Chromosomal analysis was successful in 318 cases. All samples were tested for in vitro cytotoxicity for fludarabine, AMSA, mitoxantrone, vepeside, daunobucine and Ara-C after 4days culture, using the ATP assay, in vitro cytotoxicity was correlated to chromosomal aberrations. In the 318 patients, five main groups were identified: cases with monosity 7 or deletion 7q (n=52); complex karyotype (n=50); normal karyotype (n=114); abnormal chromosome 17 (n=20) or abnormal 9p (n=15). Complex karyotype and the chromosome 7 abnormalities are well known markers for poor prog-
nosis, long term outcome and in vitro drug resistance. The first three groups were compared to patient’s samples with abnormal chromosome 17/9p. Results. Abnormalities of chromosome 17 indicate a significantly higher drug resistance for all drugs tested and a significant shorter overall survival compared to patients with normal and complex karyotype. A shorter overall survival and higher drug resistance was also noted when comparing samples to therapy and adverse outcome, even when compared to other high risk karyotypes in AML. Patients with abnormalities in 9p, which affect p53 protein pathway and degradation, showed a shorter overall survival but less obvious drug resistance.

**0126 LEUKEMIA IN UKRAINIAN CLEAN-UP WORKERS OF THE CHORNOBYL ACCIDENT: EPIDEMIOLOGIC AND HEMATOLOGIC ASPECTS**

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Background. Leukemia holds a special place in the study of radiation-related cancer because bone marrow is one of the tissues most sensitive to the carcinogenic effect of ionizing radiation, radiogenic leukemia has the shortest latent period among radiation-induced cancers, and its appearance is a sign of solid tumor appearance. U.S. National Cancer Institute (study team - A Bouville, M.Hatch, G Howe, N Luckyanov, I Masnyk, I Zablotska) and Research Center for Radiation Medicine from Ukraine (study team NG Bakhkina, E Bakhanova, El Bomko, Yu Byelyayev, V Chunak, I Dyagil, NA Gudzenko, TF Lubarets) have initiated a study of radiogenic leukemia. Aims. The main objective is to test the hypothesis that exposure to radiation during cleanup operations following the Chornobyl accident led to an increase in leukemia among male clean-up workers from Ukraine. Results. A retrospective case-control study of ionizing radiation and leukemia was conducted in a cohort of 110,645 male Ukrainian liquidators involved in cleanup work following the accident at the Chornobyl nuclear power plant in northern Ukraine which occurred on April 26, 1986. The cohort includes 46% of clean-up workers in Ukraine. Information on all cases from 1986 to 2000 was collected in the hematological, pathological departments of local hospitals and registries of radiation exposed after Chornobyl in 5 target areas of Ukraine and Kyiv city including clinical records and blood smears, bone marrow slides, cytochemical and histological preparations, immunophenotype. Cases were evaluated by the international diagnostic scheme from 5 pathologists (2-USA, 2-Ukraine, 1-United Kingdom). Annual case distribution showed a marked tendency to increase with age after the exposure. To assess the influence of ionizing radiation exposure analysis was performed of observed and expected (spontaneous) leukemia numbers at the cohort basing on the data on male population of Ukraine. The doses in cases obtained by RADRUE retrospective dosimetry were higher than in controls. Risk estimates were performed. Summary. Obtained data show the elevation of cases number and radiation risks in Chornobyl clean-up workers in Ukraine 8-14 years after the radiation exposure. Further follow-up of the cohort is performed.

**0127 QUANTITATIVE ASSESSMENT OF AML/ETO FUSION TRANSCRIPT AS USEFUL TOOL FOR MINIMAL RESIDUAL DISEASE DETECTION AND OUTCOME PREDICTION IN T(8;21) AML**

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Background. The t(8;21) translocation derives from the fusion of AML1 on chromosome 21 and ETO on chromosome 8. It is associated with FAB subtype M2 acute myeloid leukemia (AML). In order to predict relapse, qualitative PCR has a limited value since a positive PCR can be observed for a long follow-up period, even during continuous complete remission (CCR). From preliminary studies, quantitative RT-PCR seems to be a good candidate to predict clinical outcome of patients presenting AML1-ETO rearrangement. Aims. To test the usefulness of quantitative RT-PCR assay in detecting minimal residual disease and in predicting relapse in patients affected by t(8;21) AML. Methods. We analyzed in a retrospec-

manner 123 PB and BM samples from 33 patients affected by AML presenting AML1-ETO rearrangement by standard nested RT-PCR. 111 out of 123 BM samples were also analyzed by quantitative RT-PCR technique following standard Methods. Samples were taken at diagnosis, after induction and consolidation therapy. Results. The median age of the patients was 54 years (range 6-60). 13 patients underwent conventional therapy and 20 were treated with autologous or allogeneic bone marrow transplantation. WBC median value at diagnosis was 7905 (range 400-23300). 9 out of 21 patients who achieved and remained in CCR showed qualitative RT-PCR positivity after consolidation treatment, in two cases even after autologous and autologous transplantation showing a late clearance of the transcript. By contrast, 5 patients out of 12 achieved PCR negativity but subsequently relapsed. We compared the values obtained by RT-PCR in the 21 patients who achieved complete remission to the 12 who relapsed during follow-up. At diagnosis quantitative analysis of AML-ETO fusion transcript showed a large variability (median value 71500, range 9632-801900). No difference was found between transcript amount at diagnosis and clinical outcome (p=0.6 by Mann-Whitney test). We did not observed any significant correlation between transcript amount and either WBC values (r=0.14) or blast percentage (r=0.25). Values obtained after induction treatment are not different in patients who reached CR as compared to those who relapsed (p=0.17). By contrast, after consolidation treatment, patients in CR showed a median transcript level of 14, compared to 395 AML-ETO copies in those who subsequently relapsed (p=0.014). We also tried to identify a threshold level of transcript after consolidation to identify the CCR patients. We can observe that only two patients out of 21 in CCR reached a post consolidation value > 15 copy numbers. On the other hand, 11 patients with a consolidation obtained a post consolidation value below 15 copies subsequently relapsed. Conclusions. Quantitative RT-PCR assessment of AML/ETO fusion transcript amount in leukemic patients is a useful tool for detecting minimal residual disease. Our data demonstrated that the most significant value to predict final outcome is the one obtained after consolidation treatment. We can also identify 15 AML-ETO copies after consolidation as threshold level in order to predict patients’ outcome.

**0128 PROGNOSTIC SIGNIFICANCE OF BAALC EXPRESSION IN ACUTE MYELOID LEUKEMIA WITH NORMAL KARYOTYPE: FROM MICROARRAY TO RTPCR ASSAY**

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Background. Clonal cytogenetic abnormalities are one of the most important factors predicting clinical outcome in Acute Myeloid Leukaemia (AML) and are used to guide risk-adapted treatment strategies. However, approximately 50% of de novo AML have normal karyotype and therefore lack informative chromosome markers. The identification of relevant genetic features as well as the discrimination between different subsets of patients within this group remain major challenges. BAALC high expression in pre-treatment blood samples (peripheral blast) was proposed to be an independent adverse prognosis factor for Overall Survival (OS) Event Free Survival (EFS) and Disease Free Survival (DFS). Aims. The goals of this study were to determine if expression of BAALC was a prognosis factor in AML by using a new specific quantitative PCR (RQ-PCR) assay and to compare these results with data obtained on microarrays. Methods. We developed a new specific RQ-PCR assay for quantification of BAALC transcripts in human cells. This assay is based on the plasmid technology which allows the precise control of RNA amplification and normalization of RQ-PCR results and is highly reproducible. The ratio of BAALC transcript to endogenous ABL1 transcript provides a normalised quantification of BAALC independent of the cell count and RT efficiency. We previously profiled on a 9000-cDNA microarray 61 adult AML samples with normal cytogenetic at diagnosis. Prognostic significance of BAALC and EVI1 expression levels was analysed using univariate Cox proportional hazards regression analyses. Results. Microarray data showed that BAALC expression values were associated with outcome (p=0.07). In addition, we could note that when combined with EVI1 expression values, results were even better. We Thus could determine that no EVI1 expression was a more significantly correlated (CCR: BAALC+0.03; (ii) BAALC+ and BAALC+/EVI1- patients correspond to a poor prognosis class and (iii) BAALC+/EVI1+ patients to a good prognosis class (increased OS, p=0.0076) (Figure 1). RQ-PCR assay analytical validation showed a reproducible sensitivity greater than 10-5 (less than 100 BAALC positive KG1a cells in

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5.106 BAALC negative K562 cells or 50pg of KG1a RNA in 1ug of MV4-11 RNA. We determined BAALC levels in normal peripheral blood samples (n=26). The median normalized copy number (NCN) of BAALC was 2210 and ranged between 129 and 6427. Pathological values were assessed on a subset of AML samples (n=24) already used for microarray analysis. The median NCN was 1693, and ranged between 51 and 1859. The RQ-PCR results demonstrate a clear direct correlation (p=0.0057) between OS and expression of BAALC alone in normal karyotype AML patients (Figure 2). Conclusions. We confirmed high expression of BAALC as an adverse prognostic factor for normal karyotype AML patients, and identified a BAALC - / EVI1 + class with favourable outcome. The sensitivity and dynamic range of the BAALC RQ-PCR assay may contribute to treatment option decisions at diagnosis. Large patient cohort and clinical trials will evaluate the impact of this new molecular tool on patient care. Further RQ-PCR studies will be performed to accurately assess the importance of EVI1 expression correlation with outcome in BAALC - group.

**Figure 1. Microarray data analysis, OS and DFS.**

**Figure 2. RQ-PCR data analysis.**

**0129**

**A MULTICENTER PROSPECTIVE RANDOMIZED STUDY OF LENOGRASTIM IN CONSOLIDATION CHEMOTHERAPY FOR ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA (IALSG GML200-9 STUDY)**

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**Background.** Although G-CSF administration after consolidation chemotherapy is recommended in some clinical guidelines, effective use of G-CSF administration is under investigation. Aims. We conducted a multicenter prospective randomized study to compare two different administration schedules of lenograsit in consolidation chemotherapy for elderly AML patients. The primary endpoint of this randomized study is to compare the duration of fever (above 38.5°C). Methods. Patients newly diagnosed untreated de novo AML older than 60 years of age who attained complete remission (CR) were eligible for this study. After consolidation chemotherapy with both cytarabine (200 mg/m², d1-5) and mitoxantrone (age<70 years; 7 mg/m², d1-5, age ≥70; 5 mg/m²; d1-5), patients randomly assigned to receive lenograsit (5 µg/kg, 30 min i.v.) either after absolute neutrophil count (ANC) less than 1000/µL (Arm A: prophylactic administration) or after ANC less than 1000/µL with fever above 37.5°C (Arm B: therapeutic administration) until neutrophil recovery. Results. Between August 2000 and March 2005, 110 evaluable patients were registered. 54 patients were in Arm A and 56 in Arm B, and the median age of both groups was 71 years old. There were no significant differences in patient characteristics. All patients received lenograsit in Arm A. Twenty-nine patients (51.8%) received lenograsit in Arm B, because 27 patients did not experience fever above 37.5°C. The duration of fever was not significantly different between Arm A and Arm B. The mean±SD of the duration of fever was 7.3±2.1 days in Arm A and 14.5±3.8 days in Arm B (p=0.002). The duration of infectious complications was slightly shorter in Arm A (p=0.032), the duration of fever was significantly shorter in Arm A (p=0.001). In 65-69-year-old patients who received more intensive chemotherapy, the duration of fever was significantly shorter in Arm A (p=0.05). We confirmed high expression of BAALC as an adverse prognostic factor for normal karyotype AML patients, and identified a BAALC - / EVI1 + class with favourable outcome. The sensitivity and dynamic range of the BAALC RQ-PCR assay may contribute to treatment option decisions at diagnosis. Large patient cohort and clinical trials will evaluate the impact of this new molecular tool on patient care. Further RQ-PCR studies will be performed to accurately assess the importance of EVI1 expression correlation with outcome in BAALC - group.

**Figure 1. Microarray data analysis, OS and DFS.**

**Figure 2. RQ-PCR data analysis.**

**0130**

**INTERPRETATION OF INTERLEUKIN-2 RECEPTOR Α POSITIVE CELLS DURING INDUCTION CHEMOTHERAPY FOR ADULT ACUTE MYELOGENOUS LEUKEMIA PATIENTS**


**Background.** CD25 represents IL-2 receptor α (IL-2Rα). CD25 antigen expression has been observed mainly in FAB-M4 and -M5 subtypes of acute myelogenous leukemia (AML). However, questions about the dependence or independence of both CD25+ leukemic cell functions in AML and the varying expressions of IL-2R for malignant myeloid cells remain unanswered. Unlike solid tumors, hematological diseases such as AML have demonstrated a wide range of soluble IL-2 receptor levels, which suggest the possibility of different patterns of proliferation of leukemic cells. In addition, AML cells possess the characteristics of cell surface CD25 inducibility. Aims. An attempt was made to correlate clinical outcomes with specific patterns of the expression of immune cells after induction chemotherapy (IC) in adult patients AML. Methods. Seventy-five newly diagnosed AML patients received the same initial IC and serial bone marrow (BM)- or peripheral blood (PB)-samples were taken. The gated CD45/CD25/CD4 cell populations were used to compare for the intensity of immunophenotypic signals and the different cell subsets, according to the treatment timeline. Results. As one of the best predictive prognostic parameters, patients who responded poorly to IC showed exceptionally higher levels of PB CD45+CD25+ cells on days 7 (p=0.002) and 21 (p=0.05) post-IC. The results of patients in complete remission (CR)(n=61), as well as those of the patients who showed continuous CR, showed relatively lower levels of PB CD45+CD25+ and higher levels of BM CD45+CD25+ higher CD4+CD25+ regulatory T cells in the steady PB after the standard IC, which was accurately discernible in every patient and in normal healthy individuals (n=21). We found considerably lower expression levels of BM PB CD4+CD25+ regulatory T cells in the patients. Conclusions. These findings suggest that we can use the CD25+ cells during induction chemotherapy as to predict the outcome of adult AML patients. Further, it is necessary to reveal the exact function of those emerging cells during chemotherapy in the future.

**0131**

**INHIBITION OF FLT3-ACTIVATING MUTATIONS MAY NOT PREVENT CONSTITUTIVE ACTIVATION OF ERK/AKT/STAT PATHWAYS IN SOME AML SAMPLES: A POSSIBLE CAUSE FOR THE LIMITED EFFECTIVENESS OF THERAPY WITH SMALL-MOLECULE INHIBITORS**

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AML cells are characterized by genetic alterations involved in the expression of transcriptional regulators that are critical for normal hematopoietic development and differentiation. Activating mutations on Flt3 receptors are the most common genetic alterations in AML, conferring a poor prognosis and decreased overall survival. Thus, Flt3 is now a promising target for therapeutic intervention, and a group of new small molecule inhibitors targeting the Flt3 RTK are currently being evaluated in clinical trials. However, clinical responses in relapsed or refractory AML are limited and transient. This study investigates the rele-

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vanance of activating mutations of Flt3 on the constitutive activation of intracellular pathways aberrantly active in AML, and their effect on blast cell survival. A total of 28 patients with acute myeloid leukaemia (AML) diagnosed according to the classification of the French-American-British (FAB) committee, as well as ten healthy controls, were entered into this study after informed consent. Blast cells were obtained either from peripheral blood or bone marrow aspirates. FLT3 receptor gene amplification and FLT3 gene mutations were identified by allele-specific polymerase chain reaction (PCR). Amplification of Flt3 cDNA, followed by agarose gel electrophoresis analysis. The status of activation of the MAPK/erk/STAT pathways was analyzed by both Western blot and electrophoretic mobility shifts assays. For the detection of FLT3 gene mutations, PCR amplification and DNA sequencing were performed. The FLT3 receptor gene amplification was detected in 11 of 23 patients (48%) as opposed to 6 out of 28 (21%) for FLT3-negative ones, p = 0.03. FLT3 mutations were detected in 16 of 28 patients (57%), while FLT3 D835 mutations were detected in 8 of 28 patients (29%). One patient (1%) was found as having both abnormalities. Pre-transplant therapy consisted of ICE as induction (idarubicin + cytarabine + etoposide) followed by NOVIA (mitoxantrone + intermediate dose cytarabine) as consolidation and mobilizing regimen for patients aged up to 60 years, and of continuous infusion (c.i.) of fludarabine and cytarabine (cFlA) as induction and consolidation for patients >60 years. Conditioning regimen was a combination of 5 days c.i. idarubicin plus busulfan for 4 days (I-Bu) for 56 patients (reduced by one day for both drugs for patients >60 years), and classical Bu-Cy for the remaining 17 cases. Results. Analysis of basal characteristics of the patients showed that white blood cell count (p = 0.009), serum concentration of lactate dehydrogenase (p = 0.01), and percentages of peripheral blood (p = 0.002) and bone marrow blasts (p = 0.08) were significantly higher in patients positive for FLT3 mutations. On the contrary, overall survival rates of patients with or without FLT3 mutations (p = 0.73 and 0.78, respectively). Conclusions. Our data suggest that myeloablative chemotherapy supported by auto-PBSCT may overcome the adverse prognostic implications of FLT3 mutations in AML, however, it is to consider that autografted patients are highly selected for best response to induction, consolidation and mobilization as well as for minor non-haematologic toxicity.

0134

PEDIATRIC RELAPSED ACUTE MYELOID LEUKEMIA IN THE NETHERLANDS FROM 1980 UNTIL 2000

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The prognosis of pediatric AML has improved considerably over the past decades, with overall long-term survival rates up to 60%. However, relapse remains the major cause of treatment failure, occurring in 30-40% of patients. Patients with relapsed AML have a poor prognosis. Studies from different groups have shown survival rates of 15-50%. The outcome of Dutch pediatric relapsed AML patients is unknown. We therefore studied all pediatric de novo AML patients initially diagnosed auto-PBSCT, the remaining 21 patients (29%) included early relapse (n = 10), toxicity after consolidation (n = 5) and failure to mobilize Pbsct (n = 6). Of note, early relapse rate was higher for FLT3- patients (5 out of 7, or 71%) as opposed to 5 out of 14 (36%) for the group of FLT3- patients, p = 0.27. Conclusions: the analysis of our data demonstrate that the presence of FLT3 mutations has no influence on mobilization and collection of CD34+ cells as well as on overall feasibility of PBSCT in AML patients with normal karyotype.
between 1980 and 1998 who suffered from a relapse. Most were initially treated on the subsequent Dutch Childhood Oncology Group (DCOG) studies ANLL 80, 83, 87 and 94. Data were collected from the central data collection center of the DCOG. From 1980-1998 354 patients were diagnosed with de novo AML in the Netherlands. 113 patients (52%) relapsed between 1980 and 2000. Most (65%) relapsed within a year after reaching first complete remission (CR1) (median time to relapse 9 months, range 1-56 months). 80% (N=90) of patients were within a year after reaching first complete remission (CR1) (median time was 0.16 (SE=0.04). Patients that relapsed early (CR1<1) were significantly less likely to survive than patients that suffered from a late relapse (5-year p0.01 vs. 0.29, p<0.0001). One third (8/22) underwent an autologous and two-thirds (14/22) an allogeneic SCT. We performed multivariate analysis, including SCT (as a time-dependent variable) and CR1 duration (CR1<1 year). Both SCT and CR1 duration were significantly correlated to survival. Patients that received a SCT in CR2 had a significantly improved survival (RR=0.43, p=0.008), while a CR1 duration ≤1 year resulted in a significantly poorer survival (RR=2.7, p<0.0001). In conclusion, relapse duration and SCT patients are longitudinally alive survivors. Patients with an early relapse do worse compared to patients with a late relapse, confirming results from other study groups. There is a survival benefit for SCT after relapse. We recently opened an international randomized phase III trial for children with relapsed/refractory AML (I-DFM/DCOG Relapsed AML 2001/01), randomizing FLAG (flu- darabine, cytosine arabinoside, daunorubicin, and G-CSF) with or without liposomal daunorubicin. This ongoing trial enables internationally uniform treatment of the rare cases of relapsed AML in children and will show us if the addition of liposomal daunorubicin is of benefit for these children.

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Background. Protein kinase CK2 (formerly casein kinase II) is a highly conserved, and ubiquitously expressed protein serine/threonine kinase implicated in various cellular processes including proliferation, differentiation, and transcription. However, the biological significances of CK2 can not be elucidated in acute myeloid leukemia (AML). Aims. We tried to evaluate the clinical and biological significances of CK2 in AML. Methods. We first analyzed the expression and activity of catalytic subunit of CK2 (CK2α) by Western blot and its association with clinical outcomes in consecutive 59 AML patients with normal karyotype. Secondly, we performed Western blot analysis to demonstrate that CK2 expression was observed in 30 (50.8%) cases. Constitutive expression of CK2α was not demonstrated in bone marrow samples obtained from healthy volunteers. Levels of CK2α expression were highly correlated with CK2α catalytic activity (p<0.0001) in primary AML cells. Kaplan-Meier analysis showed that the disease-free survival (DFS) and overall survival (OS) rate were significantly lower in the CK2α-positive cases compared to the CK2α-negative cases (p=0.05 and p=0.001, respectively). Multivariate analysis revealed that CK2α expression was an independent prognostic factor in the DFS (p=0.002) and OS (p=0.003). Treatment of U937 leukemia cell line with a CK2-selective inhibitor, apigenin, for 24 h potentially reduced the expression levels of phosphorylated pTEN, phosphorylated Akt/PKB and Akt/PKB downstream molecules in a dose-dependent manner. In contrast, an induced overexpression of CK2α increased the levels of anti-apoptotic proteins including Bcl-2, Bcl-xL, Mcl-1, survivin and XIAP in U937 cells. Although apigenin did not potentially induce cell death in U937 cells, an induced over-expression of CK2α remarkably enhanced the sensitivity of the cells to the apigien-induced cell death. Interestingly, apigenin-induced cell death was remarkably higher in the CK2α-positive primary AML samples (82±4%) compared to the CK2α-negative AML samples (4±1%, p=0.001), or normal BM samples (5±2%, p=0.001). Conclusions. These results strongly suggest that protein kinase CK2 is an independent prognostic marker in AML with normal karyotype. In addition, our finding that CK2 inhibitor effectively induces cell death preferentially in the CK2α-positive AML provides a novel approach to the targeted therapy for AML.

0136 INTERLEUKIN-2 CAN BE SAFELY ADMINISTERED TO AML PATIENTS IN 1ST CR AFTER AN AUTOLLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANTATION S.M. Trisolini, S. Capria, L. Cardarelli, V. Gianfelici, G.A. Natale, M. Ribersani, F. Mandelli, R. Foà, G. Meloni
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Interleukin-2 (IL2) is a cytokine with anti-tumor activity. When administered after autologous stem cell transplantation, it appears to reproduce the graft versus leukemia effect of allogeneic transplant and possibly prolong disease-free survival (DFS). Since 1999, at the Hema-
tology Department of La Sapienza University in Rome, 24 AML patients treated CR underwent immunotherapy with IL2. All patients received hydroxyurea followed by HD or SD-AraC plus Daunorubicin and Etoposide as induction treatment, and Daunorubicin plus ID-AraC as consolidation. Subsequently, an autologous or allogeneic peripheral blood stem cell transplantation (PBSC) was planned according to donor availability. IL2 was administered following PBSC after BU-CY condition regimen in 18/24 patients, and after consolidation in 6 patients not eligible for an autograft because of infections (2 pts) or mobilization failure (4 pts). IL2 therapy was started after a median of 4 months from autograft (range 1-7) and a median of 7.5 months from consolidation (range 4-14). An absolute neutrophil count higher than 1x10^9/L, a platelet count greater than 50x10^9/L and absence of active infections were required to start the treatment. IL2 was administered subcutaneously on 5 consecutive days, on a monthly basis, for 1 year or until relapse. The dosage of IL2 was 4x10^5 IU on day 1, followed by 8x10^6 IU on days 2 through 5. All patients received paracetamol and proflavine/trimethoprim/sulfamethoxazole to prevent bacterial infections during IL2 therapy. No patient required treatment discontinuation because of a grade of 4 toxicity according to NCI-CTC criteria. Fever (grade 1-2) was observed in all patients 4-6 hours after IL2 administration, with grade 1 arthralgia in 15 of them. The majority of patients showed gastrointestinal toxicity (grade 1-2) in the form of nausea and vomiting (21/24), diarrhea (4/24) and transient transaminase increase (7/24). Skin toxicity (grade 1-2) was observed as desquamation (7/24), rash and pruritus which required systemic measures (4/24) and injection site reactions (16/24). With regard to hematological toxicity, grade 3 thrombocytopenia requiring a 50% dose reduction was observed in 2 patients. Concerning neurological toxicity, only 2 patients showed irritability and insomnia during IL2 administration, not requiring dose modification. No patient showed infective complications. In all cases, toxicity completely recovered within 48 hours from IL2 discontinuation. Five patients relapsed on therapy, after 2 (CNS relapse), 3, 4, 6 and 11 months from the start of IL2, while 2 patients relapsed after 5 and 18 months from treatment discontinuation. One patient is still on therapy and 16 are in CCR after a median of 18 months (range 1-63) from IL2 discontinuation. The eighty-months projected probability of DFS for the 24 patients is 55%, the median has not been reached. Based on our experience, it appears that IL2 therapy is a feasible approach devoid of serious toxicities when administered after an autograft, as an ongoing trial is currently ongoing in the context of the EORTC/GIMEMA AML12 protocol to document whether or not IL2 is capable to enhancing the likelihood of disease-free survival.
AML. However, the success of stem cell MRD will increase using anti-CD34+CD38- population in some patients, and to the possibility to assess rate for (IDS) and palmitoylated membrane protein (P55) genes at the RNA level. AML at remission were selected. A non radioactive reverse transactivation, but aberrant methylation is known to occur in some situations. (AML). Previous studies have used DNA methylation to measure X inactivation during the remission in AML patients at the RNA level. Aims. (CD34+/CD38-/CD45dim/SSClow/aberrant marker(s)], stem cell MRD from the introduction of the alternative stem cell population, i.e. the side channels. The main impr.

Results. For comparison, whole blast MRD measurements using LAP was possible in 51/60 CD34-positive patients. In 3 patients without whole blast MRD possible, stem cell MRD could be used. The higher success rate of whole blast MRD is partly due to the absence of a detectable CD34+CD38- population in some cases and to the possibility to detect LAPs on the CD34 negative compartment in the CD34 positive AML. However, the success of stem cell MRD will increase using antibody combinations known as LAP, but which need at least 5 fluorescence channels. The main improvement in stem cell MRD will likely come from the introduction of the alternative stem cell populations, i.e. the side population (SP). In 7/7 AML samples studied thus far and including both CD34-positive samples and CD34-negative samples, SP cells were present and shown to have LAP/CLL-1 expression in all cases (see abstract Moshaver et al.). Lastly, since the population of interest is so well defined, both CD34/CD85j-CD45dim/SSClow/aberrant marker(s)], stem cell MRD may require much less expensive experience than whole blast MRD.

ANALYSIS OF X-CHROMOSOME INACTIVATION PATTERNS IN IRANIAN PATIENTS WITH AML DURING REMISSION

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Background. Analysis of x-chromosome inactivation patterns in female patients have been used to assess clonality of various tumours and X-linked disorders. Conflicting results have been published on the frequency of clonal patterns in female patients with Acute Myeloid Leukemia (AML). Previous studies have used DNA methylation to measure X inactivation, but aberrant methylation is known to occur in some situations. Aims. The aim of this study was to evaluate patterns of X-inactivation in the remission in AML patients at the RNA level.

Materials and Methods. Two hundred normal females and 45 patients with AML at remission were selected. A non radioactive reverse transcription polymerase chain reaction (RT PCR) method was used to study the expression of the polymorphisms of G6PD, iduronate 2-sulfatase (IDS) and palmitoylated membrane protein (P55) genes at the RNA level. Results. The frequency of heterozygosity was found to be 48.5% (119/245) for P55 gene. Forty percent (93/245) were heterozygous for IDS and only 28.9% (71/245) of individuals showed polymorphism at 11Zanjan Medical School, ZANJAN, Iran; 2Tarbiat Modares University, TEHRAN, Iran; 3Pescara Hospital, PESCARA, Italy; 4Tor Vergata University, ROME, Italy; 5Foggia Hospital, FOGGIA, Italy; 6Catholic University, ROME, Italy.

Background. The acute myeloid leukaemia (AML)-M4 subtype is frequently associated to eosinophilia and/or to the cytogenetic alteration inv(16). The presence of eosinophilia in AML can be associated with good prognosis, but the studies concerning their exact role are hampered by the low number of cases. Aims. To assess the influence of eosinophilia and of the inv(16) on the prognosis of acute myelomyelocytic leukaemia (M4) and acute myelomonocytic leukaemia with abnormal eosinophilia (M4Eo). Methods. In a non concurrent-prospective setting, we analyzed patients with AML-M4 consecutively enrolled in two GIMEMA clinical trials, in 35 Italian hematological divisions. Results. Between December 1998 and December 2002, 1636 valuable adult patients over 1702 with a diagnosis of AML, were consecutively admitted to the EORTC-GIMEMA AML10 and AML 99p trials; among these, 400 patients (205 M4 and 45 M4Eo) were studied. The diagnosis of M4 and M4Eos was first established at each institution and subsequently centrally reviewed at the time of study entry. The following parameters were evaluated: morphology, immunophenotype, cytogenetics performed at the onset of the disease, complete remission achievement and duration, overall survival (OS) and disease-free survival (DFS) from AML diagnosis. Cytogenetic analysis failed or was not carried out in 40% of cases, while it was successfully analyzed in 240 cases; inv(16) was found in 17% of them. Patients with M4Eo were younger and more frequently associated with inv(16) compared to M4. Concerning the probability of obtaining a CR after standard treatment, at univariate analysis M4Eo had a trend significant advantage compared to M4, while presence of inv(16) was significantly correlated to a higher CR probability; the proportion of patients with resistant disease was higher in patients with M4 morphology compared to M4Eo. Fitting a statistical model for the analysis of factors including interactions, the multivariate analysis showed a significant advantage only of M4E0 + inv(16) compared to M4 without eosinophilia and without inv(16). DFS was not different in univariate analysis between patients carrying or not inv(16), while a borderline advantage of M4Eo was observed with respect of M4, not confirmed at multivariate analysis. OS curves showed at univariate analysis a significant advantage for patients without the presence of eosinophilia (p=0.004) and of inv(16) (p=0.01); at multivariate analysis, patients with M4Eo+ inv(16) had a highly significant advantage compared to M4 without eosinophilia and without inv(16) (p=0.004), but also compared to M4 + inv(16) (p=0.043), and M4Eo without inv(16) (p=0.076). Finally OS and DFS of the 400 patients with M4and M4Eo was compared to the general AML population with the median of the cases of M4 and M4Eo was compared to the general AML population with the median of the cases of M4 and M4Eo was significantly associ-

0138

POLYMORPHISMS IN THE RAD51 AND XRCC-3 GENES INCREASE THE RISK OF DEVELOPING ACUTE MYELOID LEUKAEMIA

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Background. DNA is at constant risk from damage from both endogenous and exogenous sources and this damage causes chromosomal instability leading to oncogenesis, apoptosis and severe failure of cell functions. DNA is protected from damage by detoxification enzymes belonging to two different classes or damage triggering phases: phase I enzymes metabolise the exogenous agent to a reactive state, whereas phase II
enzymes detoxify the reactive intermediate by catalyzing conjugation. Even if greater activation and lesser detoxication of carcinogenic result in DNA adducts, it is still possible for genomic integrity to be restored through DNA repair. Several polymorphisms in genes involving in both detoxification and repair pathways have been identified and many of them have been shown to influence the risk of developing solid tumours and haematological malignancies. Aims: the aim of our study is to investigate the frequency of polymorphisms involved in detoxification and double strand break (DSB) repair via homologous recombination (HR) pathways and to correlate them with AML or therapy-related AML (t-AML) risk. Methods: we studied 160 patients with AML (132 de novo and 28 therapy-related) and 154 control subjects, matched for age and sex. RFLP PCRs were used to analyze genotypes of DNA repair genes for RAD51 (RAD51-G185C) and XRCC3 (XRCC3-Th241Met) and detoxification genes for NQO1 (NQO1-Pro187Ser) and GSTA1 (GSTA1 promoter region, A*B). The polymorphism in the promoter region of detoxification gene CYP3A4 (CYP3A4-A290G) was examined by mismatch PCR. Results: When comparing AML patients to controls, a statistically higher prevalence of the g/c + c/c genotype of the DNA repair enzyme RAD51 was found in AML patients (22.6 vs 12.7%, OR 1.9, 95% CI 1.36-3.35, p=0.04), in particular when associated to the CYP3A4-A290G detoxification enzyme polymorphism. This was confirmed by the multivariate analysis (p=0.047 for the association). Similarly the homoygous met/met mutant of XRCC3 was more frequent in AML patients (24% vs 12%, OR 2.3, 95% CI 1.2-4.3). No differences were found when looking at NQO1, GSTA1 and CYP3A4 polymorphisms, alone or in association to XRCC3. In the AML patient group, we found no associations between enzymatic polymorphisms and type of AML (de novo versus therapy-related). Summary/Conclusions: DNA repair and enzymatic polymorphism in the RAD51 and XRCC3 genes may increase the risk of developing acute myeloid leukaemia. This risk is particularly high when the RAD51-G185C DNA repair polymorphism is associated to the CYP3A4-A290G detoxification enzyme polymorphism.

0141
THE ANTI-PROLIFERATIVE AND APOPTOSIS-INDUCING EFFECTS OF THE PROTEASOME INHIBITORS BORTezOmbi AND PR-171 IN ACUTE MYELOGENOUS LEUKAEMIA
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Background. Proteasome inhibitors represent a new class of anti-neoplastic drugs with documented effects in multiple myeloma and mantle cell lymphoma. in vitro studies suggest that these drugs also have effects on other haematological malignancies. Aims. The aim of this study was to investigate the in vitro effects of proteasome inhibitors on human primary acute myelogenous leukaemia (AML) blast proliferation, viability/apoptosis induction and clonogenic potential. Furthermore, possible correlations with genetic features of AML cells, such as Fhit mutations and cytogenetic abnormalities were assessed. Methods. Native human AML blasts from peripheral blood of more than 50 consecutive patients were analysed. The impact of proteasome inhibition was examined in the following experimental models: - Cell proliferation: Leukaemia cells were cultured in vitro in the presence of exogenous growth factors (IL-3, SCF, GM-CSF) for 7 days and cell proliferation was measured by either [3H]-thymidine incorporation or a clonogenic assay. - Cell viability: Leukaemia cells were cultured in the presence (7 days) or absence (2 days) of exogenous cytokines and apoptosis/viability was measured by flow cytometry analysis of Annexin-V expression/Propidium iodide exclusion. - Cytokine secretion: ELISA and Multiplex assays for CXCL10, CCL3, CXCL8, GM-CSF, IL-1β, IL-6 and TNFα. - Proteasome activity: release of the Fluorophore 7-aminom-4-methylcoumarin (AMC) from N-Suc-Leu-Leu-Val-Tyr-AMC. We investigated the reversible proteasome inhibitor bortezomib (Velcade®) and a novel epoxomicin derivative, PR-171, which is an irreversible inhibitor (Demo SD, et al. Biochemical and cellular characterization of the novel proteasome inhibitor PR-171 [abstract]. Blood 2005; 106:485s). Results: Basal proteasome specific activity in AML blasts varied 1-10 fold. Both drugs inhibited proteasome complex with equivalent potency and suppressed cell viability as determined by annexin/PI staining after 48 hr compound treatment (% Annexin/PI negative cells; mean with standard deviation: control 33±5; bortezomib 25nm 9±5 and 50nm 12±11; PR-171 25 nm 9±5 and 50 nm 6±9). The drugs also inhibited AML cell proliferation as measured by incorporation of [3H]-thymidine after 7 days of compound treatment (IC50 95% confidence intervals: bortezomib 20.99-24.78 nM; PR-171 9.76-10.43 nm). Cytotoxic and antiproliferative responses of blasts to proteasome inhibition were heterogeneous, but the sensitivity of blasts to PR-171 was associated with the DNA repair status of the cell line. A subset of AML patient samples exhibited greater sensitivity to PR-171 relative to bortezomib in both [3H]-thymidine incorporation and colony formation assays. Both drugs modulated the constitutive cytokine release by human AML cells. Conclusion. Our studies show that proteasome inhibitors have dose-dependant and marked effects on proliferation, viability and clonal growth inhibition properties of human native AML blasts at nanomolar levels in vitro.

0142
SINGLE CELL ANALYSIS OF PHOSPHOINOSITIDE 3-KINASE/AKT AND ERK ACTIVATION IN ACUTE MYELOID LEUKEMIA BY FLOW CYTOMETRY
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Background. Acute myeloid leukemia (AML) is an aggressive malignancy and new therapeutic agents are needed. Abnormal activation of several signal transduction pathways such as phosphoinositide 3-kinase (PI3K) and MAP kinase has been reported in AML. To test new targeted therapeutics, it is critical to develop sensitive analytical tools to detect activation of these pathways and to monitor their inhibition in response to treatment. Aims. Our aim was to establish the feasibility of a flow cytometry analysis in patients samples even with a low blast infiltration and to analyze the correlation between flow cytometry and western blot analysis (WB). Methods. We analyzed AKT and ERK phosphoproteins in blast cells of 72 patients with acute myeloid leukemia by flow cytometry (WB). For the CD34 positive AML, we set up a four color protocol with CD45 and CD34. Using CD45 allowed to identify the blast cell population even in CD34 negative AML (one third of the patients in our experience). For the CD34 positive AML, we used a four color protocol with CD34, CD38 and CD123 as membrane antigens. This protocol allowed us to detect phosphorylated proteins in the most immature leukemic cells with the CD34+CD123−/low CD123+ phenotype. Results. The correlation between flow cytometry and WB was excellent. In our series we detected PI3K and ERK pathway activations in 45% and 70% of the samples. Flow cytometry allowed the analysis of samples that were not interpretable with WB. In the positive samples with high blast infiltration we compared WB and flow cytometry techniques. The flow cytometry protocol associated intra cellular staining for phospho proteins and membrane staining for several antigens including CD45 and CD34. Using CD45 allowed to identify the blast cell population even in CD34 negative AML (one third of the patients in our experience). For the CD34 positive AML, we used a four color protocol with CD34, CD38 and CD123 as membrane antigens. This protocol allowed us to detect phosphorylated proteins in the most immature leukemic cells with the CD34+CD123−/low CD123+ phenotype. Results. The correlation between flow cytometry and WB was excellent. In our series we detected PI3K and ERK pathway activations in 45% and 70% of the samples. Flow cytometry allowed the analysis of samples that were not interpretable with WB. In the positive samples, we could identify an immature blast cell population among the whole leukemic bulk that already harbored PI3K and ERK activation. When we detected phosphorylated proteins in the whole blast cell population, this activation was already present in the most immature cells, that represent exquisite target cells for new therapeutics.
ABERRANT EXPRESSION OF CELLULAR RETINOL-BINDING PROTEIN-1 (CRBP-1) IN MEGAKARYOCYTES AND MARROW STROMA CELLS OF CHRONIC MYELOPROLIFERATIVE AND MYELODYSPLASTIC/MYELOPROLIFERATIVE DISORDERS


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Background. The effects of retinol (ROL) are mediated by cytoplasmic binding proteins involved in retinoid transport and/or metabolism, as well as nuclear receptors which act as ligand-dependent transcriptional regulators. Cellular retinol-binding protein (CRBP-1) contributes to the esterification of ROL to retinyl esters, the oxidation of ROL to retinal, the hydrolysis of retinyl esters into ROL. It also has been implicated in several malignancies. The up-regulation of CRBP-1 in MSCs/MFs was present in subsets of MPD patients, but not in normal controls and increased from praefibrotic CIMF to CIMF III. Colocalization of CRBP-1 and SMA was documented in situ expression patterns of CRBP-1. Methods. This study was performed on a cohort of healthy bone marrow donors (n=15), patients with essential thrombocytemia (ET; n=25), chronic idiopathic myelofibrosis (CIMF; n=25), polycythemia vera (PV; n=25) and in MDS/MPD with features of so-called essential thrombocytemia with ringed leukemia (ET/RS; n=70). The tissue localization of CRBP-1 in marrow trephines was visualized using a well characterized antibody. Imaging was performed by bright-field and confocal laser scanning microscopy (CLSM). Double-labeling experiments included a panel of antibodies such as CD61, CD34 or α-smooth muscle actin (SMA). Evaluation focused on CRBP-1 expression in megakaryocytes and bone marrow stromal cells/myofibroblasts (MSCs/MFs). CRBP-1+ MSCs/MFs were present in subsets of MPD patients, but not in normal controls and increased from praefibrotic CIMF to CIMF III. Colocalization of CRBP-1 and SMA was documented by CLSM. Results. The up-regulation of CRBP-1 in MSCs/MFs was associated with an increased fibre density in the various MPD entities including CML, CIMF and PV, but not in ET. Bone marrow stromal cells from a subset of patients presenting with high platelet counts and ringed sideroblasts exhibited traits of MSCs/MFs which were similar to classical CML. Megakaryocytes from healthy control persons showed a moderate to high cytoplasmic CRBP-1 immunoreactivity. In contrast to the stroma, heterogeneous levels were demonstrated in megakaryocytes of PV and subsets of ET. CRBP-1 loss or abnormal spotty localization was most prominent in the bizarre giant megakaryocytes of CIMF while smaller megakaryocytes of CIMF showed a stronger cytoplasmic immunolabeling. Solar patterns of CRBP-1 expression were observed in a subset of patients with ringed sideroblasts with bizarre megakaryocytes. Conclusions. The modulation of CRBP-1 in MSCs/MFs of the marrow microenvironment may affect proliferation, migration, differentiation, matrix synthesis and turnover in MPD. Moreover, the retinoid-signaling cascade may be impaired in megakaryocytes. Aberrant regulation may occur as a consequence of point mutations or a disruption of the ordered pattern of DNA methylation. Until now, the molecular mechanisms affecting CRBP-1 in MPD and MDS/MPD overlap syndromes remain to be identified.
Table 1. MMR and EFS according to molecular response.

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Only molecular response at 6 months was significantly associated with EFS at 30 months: <1-log reduction 40%; <2-log reduction 66%; <3-log reduction 96%; >3-log reduction 100%. The degree of log-reduction in BCR-ABL/BCR at 5 months, and at the time of CCR was not predictive of EFS. Summary: In this study where achievement of CCR is accelerated (in comparison with IRIS), molecular response at the time of detecting CCR lacks prognostic value with regard to EFS, highlighting the importance of molecular monitoring at regular intervals, rather than waiting until CCR is achieved. Despite excellent treatment responses overall, with early molecular monitoring it is possible to identify a group of patients whose chance of achieving MMR is low, and in whom closer monitoring or alternative treatments should be considered.

0146

BCR-ABL PEPTIDE VACCINATION INDUCES ADDITIONAL MOLECULAR IMPROVEMENT IN IMATINIB-RESPONSIVE CHRONIC MYELOID LEUKAEMIA

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CML is characterised by the fusion BCR-ABL protein. The unique amino acid sequences KGSKCALQR and GFKGSSKLAL spanning the e14a2 (b3a2) fusion junction may be expressed on CML cells. In our phase I/II Evaluation of Peptide Immunisation in CML (EPIC), the vaccine consisted of 3 peptides containing 9 and 13-mer sequences spanning this region, both modified and linked to the 15-mer pan HLA-DR epitope (PADRE; to which all subjects are immunologically naive). All 15 entrants were in complete haematological (ly log reduction over the 15 months after vaccination was calculated (pre-EPIC), similarly, the mean 3-monthly log reduction over the 15 months after vaccination was calculated (post-EPIC). Of 12 assessable cases, 7 had received imatinib for more than 12 months pre-vaccination. In 6 of these 7, the post-EPIC BCR-ABL fall is greater than the pre-EPIC fall. Conversely, among the 5 cases who had received imatinib for less than 12 months pre-vaccination, the pre-EPIC BCR-ABL fall was greater than that post-EPIC in 3 cases. In summary, the data suggest that the BCR-ABL peptide vaccination can generate an immune response that drives additional molecular reductions of BCR-ABL in some CML patients. The vaccination strategy appears most effective in patients who have already responded well to at least 12 months of imatinib, where it may provide additional molecular control over that achievable by imatinib alone. However, vaccination may be ineffective in patients who have failed imatinib.

0147

P210 BCR-ABL TYROSINE KINASE INTERACTS WITH HISTONE DEACETYLASE 1 IN CHRONIC MYELOID LEUKAEMIA HEMATOPOIETIC PROGENITORS: CONSEQUENCES ON HISTONE H4 ACETYLATION AND CHROMATIN STRUCTURE

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The BCR-ABL fusion gene originated from balanced (9;22) translocation is the molecular hallmark and the causative event of Chronic Myeloid Leukaemia (CML). The interactions of its p210 protein constitutively activated and improperly confined to the cytoplasm with multiple regulatory signals of cell cycle progression, apoptosis and self-renewal induce the illegitimate enlargement of clonal hematopoiesis and genetic instability that drives the fully transformed phenotype of blast crisis. However, its effects on the basic transcription machinery and chromatin remodeling are unknown. Our study underscored histone H4 hyperacetylation associated with p210 tyrosine kinase (TK) in vitro and in vivo and its role in BCR-ABL transcription. Histone H4 acetylation status was assessed in 2D murine myeloid progenitors by using immuno-precipitated (IP) chromatin (CHIPs) with an anti-Ac-H4 antibody. Under permissive culture condition for p210 TK (33°C), histone H4 acetylation was reduced between 4 and 24 h of imatinib mesylate (IM) exposure concomitantly with p210 dephosphorylation and enzymatic activity reduction. In vivo histone H4 acetylation signals on CHIPs of CD34+ progenitors from CML patients at diagnosis were more intense than those of normal controls and were significantly reduced at day 15 of IM therapy. To address the putative p210 TK role on histone H4 methylation in vitro and in vivo advanced by mass spectrometry analyses we proved that histone H4 trimethylation at Lys20 was significantly reduced in presence of p210 TK and restored after p210 TK inhibition by IM in vitro. Histone H4 hyperacetylation associated with p210 TK in vitro and in vivo proceed, at least in part, from Hdac1 loss of function arising from its cytoplasmatic compartmentalisation by p210 TK and restored after p210 TK inhibition by IM in vitro. Histone H4 hyperacetylation associated with p210 TK in vitro and in vivo proceed, at least in part, from Hdac1 loss of function arising from its cytoplasmatic compartmentalisation by p210 TK. Indeed p210 TK is associated with histone H4 hyperacetylation at a BCR promoter region (-40 to +285) critical for BCR-ABL transcription in LAMA cell line. BCR-ABL transcript levels were reduced by approximately 30% at 4 h of IM exposure and further declined to 40% of untreated control at 24 h. Amplification signals of DNA from anti-Ac-H4 CHIPs were significantly reduced at 2 h of IM exposure and remained lower compared with untreated control up to 24 h (Figure 1, part A and B). Complementary activities are probably implicated in the control of histone H4 acetylation status relative to p210 TK.
BCR/ABL oncogenic kinase disrupts mismatch recognition and repair complex to induce genomic instability

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Background. BCR/ABL oncogenic tyrosine kinase is present in most chronic myeloid leukemia (CML) and in a cohort of acute lymphocytic leukemia (ALL) patients. BCR/ABL is responsible for malignant transformation of hematopoietic cells rendering them independent of their environment. The other, less understood, role of BCR/ABL in hematological malignancies is deregulation of DNA damage response, which results in drug resistance and genomic instability. Mismatch repair proteins (MMR) are responsible for detecting and removing misincorporated nucleotides, which escaped proofreading activity of DNA polymerases. MMR proteins assembled on the mismatch signal can lead to repair or apoptosis. Defects in expression of MMR genes leads to drug resistance and mutator phenotype, observed in different solid tumors. Aims. Deciphering the role of mismatch repair in drug resistance of BCR/ABL-transformed cells. Methods. MNNG (N-methyl-N-nitro-N-nitrosoguanidine), methylating agent was used as a genotoxic treatment. 32Dcl3 cells myeloid cell line along with p210BCR/ABL expressing counterparts and primary leukemia and normal bone marrow cells were employed. Cell viability was assessed by trypan blue exclusion and/or propidium iodide staining. Clonogenicity of parental and BCR/ABL cells after MNNG challenge was examined in semi-solid medium. Protein expression was analyzed by Western blotting. Immunofluorescence analysis of the nuclear localization of MMR proteins was performed with primary antibodies to MSH2, MSH6, MLH1 and PMS2. Mutation rate and phenotype was analyzed using TA cloning kit and sequencing. Results. Among different genotoxic agents, BCR/ABL cells were more resistant to MNNG than parental cells (as shown in viability and clonogenic tests). Parental cells and BCR/ABL expressing clones were incubated with MNNG for 4 weeks resulting in their MNNG-resistant derivatives, which may accumulate mutations in their genomic DNA resulting from methylaing activity of the drug. To investigate the mutation rate and phenotype, ouabain-resistance test was employed. The clonogenic assay revealed significantly over expressed in LAMA 84-R cells, indicating a possible involvement of several of these chaperone proteins in the mechanism of Imatinib resistance, via a possible block of bcr/abl proteosome degradation. A) A relevant number of proteins interacting with DNA and RNA (hnRNPF, hnRNPH1, hnRNPK and eIF3) were found to be more abundant or even expressed only in imatinib resistant cells. B) Structural proteins: vimentin, α tubulin, γ actin were instead significantly more expressed in imatinib sensitive cells. The identified proteins involved in cell signalling and in metabolic pathways (classes iv and v) resulted differentially expressed in LAMA 84-S and LAMA 84-R. The dominating signature was C to T, while A to G mutations was prevalent in parental cells (Figure i). In order to check the status of MMR proteins, Western blotting and immunofluorescence studies were performed. Expression of MMR proteins in BCR/ABL transformed cells was similar to parental, however immunofluorescence visualized dramatic changes after DNA damage in the nuclear co-localization of MMR proteins in BCR/ABL-transformed in comparison to normal cells. Co-localization of MSH2 and MSH6 proteins, forming a heterodimer homologous to bacterial MutS, remained similar in parental and leukemia cells upon MNNG treatment. However, co-localization of MLH1 (which form a heterodimer with PMS2 homologous to bacterial MutL) and MSH2 was detected in non-transformed cells, but not in BCR/ABL leukemia cells. Interaction of MMR proteins in leukemia cells was restored after inhibition of BCR/ABL kinase by imatinib. Summary/Conclusions. BCR/ABL impairs assembly of MMR proteins on mismatched nucleotides and subsequent signaling to repair and/or apoptosis. These results suggest a novel mechanism how oncogenic tyrosine kinase can modulate mismatch recognition and repair leading to genomic instability and drug resistance of leukemic cells.
Dasatinib (D) (BMS-354825) is an oral, multitargeted tyrosine kinase inhibitor of Bcr-Abl and SRC with activity against imatinib-resistant cell lines. A phase I study demonstrated preliminary evidence of activity of D in MBC-CML pts. Aims. To demonstrate the activity of D in pts who are resistant to or intolerant of imatinib. Methods. START B is an open-label study of D in IM-R or IM-I MBC carried out in 46 sites worldwide. From December 2004 to July 2005, 109 MBC pts were treated. D was given orally at 70 mg twice a day (BID) with dose escalation to 100 mg BID for poor initial response or dose reductions to 50 mg and 40 mg BID for toxicity. Pts had weekly blood counts and monthly bone marrow evaluations, including cytogenetics. Molecular monitoring of BCR-ABL transcript levels by RT-PCR were obtained at baseline and in pts who achieved response. The primary endpoint was confirmed (minimum 4 weeks duration) major hematologic response (MaHR). Results. Among the 109 treated patients, 99 were IM-R and 10 IM-I; median age was 55 yrs (range 21-81), 58% were male. Prior therapy included interferon in 53 (49%) pts, stem cell transplant in 15 (14%) pts and prior (minimum 4 weeks duration) major hematologic response (MaHR). The primary endpoint was confirmed cytogenetic response. The primary endpoint was confirmed cytogenetic response.

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ROLE OF ENDOTHELIAL PROGENITOR CELLS IN MYELOPROLIFERATIVE DISEASES


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Background. The presence of circulating hematopoietic progenitor cells has been described in patients with myeloproliferative diseases (MPD). However, the exact nature of such progenitor cells has not been specified until now. Aim: The aim of this work is to prove the hypothesis that the endothelial cell lineage is primarily involved in the pathophysiology of myeloproliferative diseases. Methods: Expression of the hematogblast markers (early common precursors to the hematopoietic and endothelial cell lineage) in the circulating cells of 53 patients with MPD was assessed. Peripheral blood was analysed for expression of CD34, prominin (CD135), KDR (kinase insert domain receptor, or vascular endothelial growth factor receptor 2, VEGFR2) and VWF (von Willebrand factor) mRNA by quantitative PCR. Clonogenic stem cell assays were performed to assess differentiation towards the hematopoietic and endothelial cell lineage. Patient data (essential thrombocythemia, ET, n=17), polycythemia vera (PV, n=21) and chronic idiopathic myelofibrosis (CIMF, n=15) were compared with data from normal controls (n=16) and patients with secondary thrombo- or erythrosis (n=17). Results: Trafficking of CD34 positive cells was increased above the physiological level in 4/17 patients with ET, 5/21 patients with PV and 3/15 patients with CIMF. A subset of patients with CIMF co-expressed the hemangioblast markers CD34, Prominin (CD135) and KDR, suggesting the presence of hemangioblasts among the circulating progenitor cells. Clonogenic stem cell assays confirmed differentiation towards both the hematopoietic and the endothelial cell lineage in 5/10 patients with CIMF. Furthermore, trisomy 8 was found in the bone marrow cells of a patient in which trisomy 8 was diagnosed in the peripheral blood, confirming the common clonal origin of both cell lineages. Conclusion: Hemangioblasts are present in the bone marrow of a subset of patients with CIMF, suggesting a primary role of pathological endothelial cells in this disease.

COMPLIANCE AND PERSISTENCY WITH IMatinib IN CHRONIC MYELOID LEUKEMIA PATIENTS

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Background. Imatinib is an oral molecularly targeted therapy with unprecedented efficacy in chronic myelogenous leukemia (CML) and gastrointestinal stromal tumors (GIST). Optimal dosing and adherence to treatment is critical to achieve the best clinical outcomes. Aim: This study examined compliance and persistence with imatinib in CML patients and identified the clinical and patient characteristics that are related to compliance and persistence. Methods: Claims data from a large US health plan were used to identify imatinib-treated patients from 6/1/01-5/31/04 who had continuous pharmacy and medical benefits in the 3 months prior and 12 months following initiation of imatinib therapy, and a diagnosis of CML (ICD-9-CM 205.1, 205.10, or 205.11). Compliance was defined by medication possession ratio (MPR=total daily supply of imatinib in the first year divided by 365). Persistence was defined as failure to refill imatinib within 30 days from the run-out date of the prior prescription. Multivariate analyses were used to identify the key factors that are associated with compliance and persistency. Results: Total 878 imatinib-treated patients were identified of whom 415 had at least 15 months’ continuous eligibility. Sixty-nine percent (n=286) were diagnosed with CML and are the subjects of this analysis. The average age was 50 (range from 3 to 86, median 50.5) and 59% were males. The average starting daily dose was 425 mg, with 81% (n=342) initiating on 400 mg daily. The mean MPR was 76%. Overall, 32% patients discontinued imatinib for at least 30 consecutive days during the 1-year follow up period. Multivariate analyses indicated MPR improved with age until age 52 and then deteriorated (p<0.001) but at a diminishing rate, decreasing by only 0.1% at higher levels of other medications used by patients (p=0.01), and was lower in women (p=0.004) and patients with more cancer complications (p=0.005). Other variables included in this analysis were starting daily dose and geographic region. In the multivariate analysis of the likelihood to discontinue imatinib for at least 30 consecutive days, women were found to be more likely to discontinue than men (OR=2.38; p=0.008) controlling for age, starting dose, cancer severity, and number of other medications used by the patient. Conclusions: Compliance to imatinib was about 75% with over 30% of patients interrupting therapy for at least 30 consecutive days in the first year. The MPR of patients with CML in this population is to be lower than the average of 97% relative dose intensity in clinical trials of imatinib (including high-dose imatinib). This suggests there may be less compliance in patients not enrolled in clinical trials. It has been found that interruption of imatinib therapy in CML may lead to rapid relapse or recurrence of disease (Cortes et al; Blood 2004; Mauro et al Leukemia Research 2004). Since compliance may affect clinical outcome, physicians should educate patients and closely monitor compliance to therapy.

COMPARISON BETWEEN CONVENTIONAL AND MOLECULARLY DEFINED THERAPEUTICS IN A CONDITIONAL BCR-ABL CELL CULTURE MODEL

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Background. Chronic myelogenous leukemia (CML) is a myeloproliferative disease in which the constitutively active tyrosine kinase BCR-ABL enhances survival of leukemic cells through modulation of intracellular signaling cascades. Accordingly, current therapeutic strategies include ABL-specific tyrosine kinase inhibitors like Imatinib (IM). However, recent studies have demonstrated drug resistance and persistence of leukemic (stem) cells under IM therapy. Therefore, combination therapy directed to a complementing target may significantly improve treatment results. We recently identified such potential targets by demonstrating that RNAi mediated reduction of SHP2, STAT5, and Gab2 protein expression inhibits BCR-ABL but not cytokine dependent proliferation (Blood. 2005 Nov 8; (Epub ahead of print)). Aim: In the current study we compared the specificity and efficacy of molecularly defined therapeutics such as IM or shRNAs inhibiting expression of SHP2, STAT5, and Gab2 with that of conventional antileukemic drugs in a conditional BCR-ABL cell culture model. Methods: We used the TonB cell line which is IL3 dependent but can be induced to express BCR-ABL by doxycycline, resulting in cytokine independent proliferation. To specifically silence expression of SHP2, STAT5 and Gab2, TonB cells were transduced with Pol III driven expression cassettes for specific shRNA transcription by lentiviral gene transfer. In addition lentiviral transgene plasmids driving the simultaneous expression of two shRNAs have been generated. Cytarabine, doxorubicin, and etoposide were used as non-specific conventional drugs. Results: We already demonstrated that inhibition of SHP2, STAT5, and Gab2 expression by RNAi specifically inhibits BCR-ABL mediated proliferation in TonB cells (Blood, in press). In contrast to shRNAs, we demonstrate here that cytarabine, etoposide, and doxorubicin inhibit both IL3 and BCR-ABL mediated cell proliferation in TonB cells. Based on the number of viable cells we determined very similar LD50 values for cytarabine (300 ng/mL, 220 ng/mL), etoposide (150 ng/mL, 110 ng/mL) and doxorubicin (4 ng/mL, 3.8 ng/mL) for TonB cells grown in the presence of IL3 or BCR-ABL, respectively. Next we combined cytarabine (300 ng/mL), etoposide (150 ng/mL) and doxorubicin (4 ng/mL) with IM. Cytotoxic drugs cooperate with IM, with complete loss of viable TonB cells in BCR-ABL mediated cell proliferation, but also 50% cell death in IL3 mediated cell proliferation. The combination treatment with shRNAs against SHP2, STAT5, and Gab2 with antileukemic drugs also resulted in more than 95% cell death in BCR-ABL mediated cell proliferation and 50-70% cell death in IL3 mediated cell proliferation. The combination of two shRNAs such as anti-BCR-ABL-anti-SHP2 and anti-BCR-ABL-anti-STAT5 or the simultaneous application of IM with anti-SHP2, anti-STAT5, and anti-GAB2 resulted in complete cell death in BCR-ABL mediated cell proliferation but no inhibition in the presence of IL-3. Conclusion: Conventional antileukemic drugs have no differential effects on BCR-ABL and IL-3 mediated cell proliferation whereas targeting of SHP2, Gab2, and STAT5 by shRNAs specifically inhibits oncogene driven proliferation of TonB cells. Combined therapy with molecularly defined therapeutics e.g. imatinib mesylate with anti-SHP2, anti-STAT5, anti-GAB2 shRNA or two shRNAs (anti-BCR-ABL-anti-STAT5, anti-BCR-ABL-anti-SHP2) cooperates in inhibiting BCR-ABL driven TonB cell proliferation with no or only minor effects on IL-3 dependent growth. Further studies on primary cells are warranted to further analyze the specificity and efficacy of combined targeted therapi
A PHASE II STUDY OF NILOTINIB (AMN107) A NOVEL INHIBITOR OF BCR-ABL ADMINISTERED TO IMATINIB-RESISTANT OR INTOLERANT PATIENTS WITH CHRONIC MYELOGENOUS LEUKEMIA IN ACCELERATED PHASE


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Background. Nilotinib is a potent, highly selective, aminopyrimidine inhibitor of Bcr-Abl which in vitro is 80-fold more potent than imatinib. This study was designed to evaluate the safety and efficacy of nilotinib as defined by hematologic and cytogenetic response (HR/CyR) rates in imatinib-resistant or intolerant AP patients. Methods. This is a Phase II open-label, multicenter, study of nilotinib administered orally at a dose of 400 mg twice daily. Results. This study remains open to enrollment. Preliminary data are reported for 22 patients (77% resistant and 23% intolerant to imatinib). The median age was 62 (range 43-76 years) and the median time from AP diagnosis was 6 months (range 0-2.5). Three of five patients with data available had a BCR-ABL mutation at baseline. The median duration of nilotinib exposure was 124 days (range 32-207). Treatment is ongoing for 16/22 (73%) patients. HR occurred in 14 (64%) of patients of which 10 (45%) were complete. Three (14%) were marrow responses/no evidence of leukemia, and 1 had a return to chronic phase. CyR occurred in 6 patients (1 complete, 1 partial, 1 minor, and 3 minimal). The AE’s occurring in ≥10% patients were thrombocytopenia (58% (n=5)), fatigue (52% (n=7)), anemia, pruritis, muscle spasms (27% (n=6)), bone pain, cough, rash (25% (n=5), diarrhea, headache, myalgia, pyrexia 18% (n=4), abdominal pain, chills, constellation, dyspnea, nausea, extremity pain, and peripheral edema 14% (n=3). The overall incidence of Grade 3/4 AE’s were thrombocytopenia 27% (n=3), anemia, neutropenia 10% (n=4 each) and rash 5% (n=1). Two deaths occurred; one from progressive disease (with thrombocytopenia had a CNS bleed) and one patient had disease progression. Summary/Conclusions. These data suggest that nilotinib is clinically active and has an acceptable safety profile when administered to patients with CML-AP.

PHILADELPHIA-POSITIVE HEMATOPOIETIC PROGENITORS ARE NOT DETECTABLE IN CML PATIENTS TREATED WITH IMATINIB ACHIEVING A MAJOR MOLECULAR RESPONSE


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Background. Imatinib mesylate can remarkably diminish the leukemic burden and produce complete cytogenetic response (CCR) in the majority of patients and some degree of molecular response. However, the clearance of the vast majority of Ph-positive cells does not necessarily translate into eradicated disease. Thus, the operational definition of complete molecular response (CMR) is the quantification of Ph-positive cells. Our data indicate that the detection of leukemic progenitors relies significantly between molecular responders and non-responders, thus suggesting a correlation between QRT-PCR and of the number of residual leukemic progenitors. Our data indicate that the detection of leukemic progenitors differs significantly between molecular responders and non-responders, thus suggesting a correlation between QRT-PCR and of the number of residual leukemic progenitors. However, since the average frequency of Ph-positive early progenitors is considerably low in CML patients at diagnosis (more than 70-80% of LTC-IC being Ph-negative) these data suggest that in patients without molecular response the impact of Imatinib on the early progenitor pool may be limited. Further studies are necessary to identify a better read-out of residual Ph-positive stem cells.

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**DASATINIB (D) IN PATIENTS (PTS) WITH ACCELERATED PHASE CHRONIC MYELOID LEUKEMIA (AP-CML) RESISTANT OR INTOLERANT TO IMATINIB: RESULTS OF THE CAL40005 START-A STUDY**

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Background. D (BMS-354825) is an oral multi-targeted kinase inhibitor with preliminary evidence of efficacy in a previously reported phase I study. Aims. To demonstrate the activity of D in patients (pts) with AP-CML resistant to or intolerant of imatinib. Methods. START A is an open-label study of dasatinib in AP-CML who were imatinib resistant (IM-R) or imatinib intolerant (IM-I). Dasatinib was given orally at 70 mg twice daily (BID). Dose escalation to 100 mg BID was allowed for inadequate initial response and reduction to 50 or 40 mg BID for persistent toxicity. Evaluation included weekly blood counts and monthly bone marrow including cytogenetics. Molecular evaluation of Bcr-Abl transcript by real-time quantitative polymerase chain reaction was performed at baseline and after documentation of complete cytogenetic response. The primary endpoint was major confirmed (maintained at least 4 weeks) hematologic response (MaHR) in IM-R pts. Results. A total of 174 pts (161 IM-R and 13 IM-I) were enrolled between December 2004 and July 2005 in 39 centers worldwide. There were 96 (55%) males; median age 57 yrs (range 22-80); median time from diagnosis of CML 82 months. Prior therapy included IM>600 mg/day in 91 (52%) pts, IM for > 3 years in 103 (59%) pts, interferon in 126 (72%) pts, stem cell transplantation in 63 (36%) pts. Overall, severe adverse events (AEs) in > 25% of pts were: cytopenia (49%), heart failure (32%), infection (29%), diarrhea (27%), and pleural effusion (11%). The most frequent were diarrhea (46%), peripheral edema (27%), pleural effusion (16%), rash (8%), and GI hemorrhage (7%). Conclusions. Dasatinib is very effective in pts with IM-R AP-CML with high rates of durable MaHR and MCyR. Data on all 174 pts will be presented at the meeting including the molecular response analysis.

**0160**

**INVOLVEMENT OF RAS, JAK2 AND GM-CSF IN THE PATHOGENESIS OF PROLIFERATIVE VARIANT OF CHRONIC MYELOMONOCYTIC LEUKEMIA**

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Background. Chronic myelomonocytic leukemia (CMML) is a heterogeneous malignancy classified among MDS/MPD disorders. The paucity of known pathogenetic events contributes to the lack of effective treatment and to its dismal prognosis. On the basis of the peripheral leucocyte count, a dysplastic subtype (MD-CMML, WBC <12×10^9/L) can be distinguished from a proliferative subtype (MP-CMML, >12×10^9/L WBC/L) of the disease. Among factors that have been implicated in pathogenesis of CMML, GM-CSF produced by either autocrine or paracrine mechanisms has been shown to be a major growth determinant. Aims. To investigate cellular and molecular differences between MD- and MP-CMML which could contribute in clarifying pathogenesis of the disease and in identifying targets for possible therapeutic applications. Methods. Peripheral blood mononuclear cells (MNC) were isolated on Ficoll-Paque density gradient from 29 patients affected by CMML (17 with MD-CMML and 12 with MP-CMML). Samples were screened for the presence of N/K-RAS genes mutations by PCR and direct sequencing. Identification of the JAK2 V617F mutation was carried out by both allele-specific PCR and amplification of exon 12 followed by restriction analysis. Also, to evaluate the expression of intracytoplasmic GM-CSF and the expression of its receptor (GM-CSFR), MNC were stained with GM-CSF PE (Caltag) and CD116 (Pharmingen), respectively. In vitro growth of myeloid colonies was assessed in semisolid medium with or without the addition of cytokines (SCF, GM-CSF, IL-3, Epo). Results. No RAS or JAK2 mutations were detected in the group of patients with MD-CMML. In the proliferative variant group, we identified two patients carrying the G12D substitution of N-RAS. Furthermore, a G60E point mutation of N-RAS was identified in 1 patient after progression from MD- to MP-CMML. The JAK2 V617F mutation was detected in 4 patients, all affected by the proliferative variant of CMML. Mean percentage of GM-CSF expression was 59.8% (range 14.5-90.7) in MF-CMML and 2.7% (range 0-9.3) in MD-CMML. The difference between MP and MD disease was statistically significant. In contrast, mean percentage of expression of GM-CSFR was similar in MD- and MP-CMML samples (40.1% vs 42.2%). However, when we considered median intensity of the GM-CSFR expression, we observed significantly higher values in MF-CMML than in MD-CMML (123.2 and 51.4, respectively). The number of CFU-GM was higher in the MP-CMML than in MD-CMML (57 vs 17/5×10^9/L cells plated) and a significant correlation with intracytoplasmic GM-CSF expression was observed (p<0.05). Conclusions. In summary, in our series of patients with proliferative variant of CMML, RAS and JAK2 mutations were quite frequent (25% and 35%, respectively). Because MP-CMML may evolve from MD-CMML, these findings support the hypothesis that molecular abnormalities could be acquired with disease progression. Moreover, since both JAK2 and RAS proteins are involved in the GM-CSF signalling pathway, the higher levels of intracytoplasmic cytokine and the increased density of its receptor in MP-CMML support the hypothesis that this pathway has a central role in malignant cell proliferation of CMML patients.
Chronic myeloid leukemia II

0162
TRANSCRIPTIONAL PROFILING OF PRIMARY IMATINIB-RESISTANT CHRONIC MYELOID LEUKEMIA
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Background. Although the selective tyrosine kinase inhibitor imatinib is successfully used in the treatment of chronic myeloid leukemia (CML), inherent mechanisms confer primary resistance in a significant minority of patients. Aims. In order to search for potentially useful genes in predicting cytogenetic response, a retrospective microarray-based gene expression study was performed. Methods. Quality-controlled RNA from leukocytes of bone marrow (BM) and/or peripheral blood (PB) of 34 interferon-alpha-pretreated chronic phase CML patients with or without major cytogenetic remission (L 35% Ph+ metaphases) during the first year of imatinib treatment was comparatively analyzed using high-density Affymetrix U133A chips. Diagnostic groups (responders vs. non-responders, n=11) were matched according to demographic and hemato logic parameters and evaluated during their clinical course for hematologic, cytogenetic and molecular responses. For the assessment of differences in gene expression, BM and PB samples simultaneously taken from seven imatinib responders were statistically analyzed based on mixed linear models. Results. Using support vector machines for gene classification, an outcome-specific gene expression signature consisting of 128 genes was identified. Comparative expression data of specific genes point to changes in apoptosis (e.g. casp9, trapl, hras), DNA repair (msh3, ddb2), oxidative stress protection (gis, pon2, vnn1), and centrosomes (pid1) within primary resistant patients. Independent statistical approaches (ANOVA, PAM) as well as quantitative real-time PCR studies on a selected subset of genes (vnn1, rph3a, tspal/b2, coch) verified the validity of the obtained data. Furthermore, the potential 128-gene predictor was tested on two independent patients with primary resistance that became accessible after having completed the study. Both test set patients were correctly assigned to their respective diagnostic group. Prospectively, our candidate predictor will be further explored on samples from CML patients currently treated in clinical studies. Conclusions. This study establishes a candidate 128-gene predictor for early assessment of primary cytogenetic response of CML patients to imatinib. The data suggest that transcriptional regulation of genes related to apoptosis, disease progression, oxidative stress, DNA repair and centrosome fidelity are associated with imatinib resistance in chronic phase CML.

0163
IMATINIB PRECEDING ALLOGENEIC STEM CELL TRANSPLANTATION IN CHRONIC MYELOID LEUKAEMIA
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Background. Little is known on Imatinib treatment prior to allogeneic stem cell transplantation in chronic myeloid leukaemia (CML). Aims. In order to investigate the outcome of allogeneic stem cell transplantation when patients were treated prior to allogeneic stem cell transplantation with Imatinib, we analyzed retrospectively the engraftment rate, incidence of acute and chronic graft-versus-host disease (aGvHD and cGvHD) and transplant related mortality (TRM) in adult patients (pts.) with chronic myeloid leukaemia (CML), who received peripheral blood stem cell (PBSC) or bone marrow transplantation (BMT) from sibling (SIB) or volunteer unrelated donors (VUD). Patients and Methods. 37 pts. (23 male, 14 female; median age 31 ± 13 years, range 16-65) were treated with SIB (n=6) or VUD (n=31) allogeneic SCT (n=11) or BMT (n=26) for CML in first chronic (CP, n=15) or accelerated phase (AP, n=17) or for more advanced disease stages: second chronic phase or blast crisis (CP2 or BC, n=7). Transplant conditioning consisted of fractionated total body irradiation (TBI) and cyclophosphamide (120 mg/kg) in all pts. All but one recipients of VUD transplants received in vivo T-cell depletion (TCD) with CAMPATH 1H (Anti CD52, n=29) or ATG (n=1). In all pts. GVHD prophylaxis was provided by Cyclosporin A and short-term methotrexate. Results. 7 pts. received Imatinib for treatment of BC, 30 pts. with the aim to achieve haematological or cytogenetic remission in CP1 or AP. The median duration of Imatinib pre-treatment was 8.5 months (range 1-31 months). The used drug dose ranged from 200mg to 800mg. Imatinib was discontinued within 8 weeks prior transplant in 26 pts. 11 pts. had a longer than 8 weeks break between Imatinib treatment and transplant. Engraftment failure occurred in 1 patient (2.7%). The transplant engraftment rate, incidence of acute and chronic graft-versus-host disease (aGvHD and cGvHD) were comparable with outcomes obtained in our institution prior to the introduction of Imatinib (2000-2005). The overall survival was 65% after a median follow up of 208 days (14-1419 days). Conclusion. In 37 pts. treated with Imatinib prior to allogeneic SCT or BMT the overall rates for engraftment failure, TRM, aGvHD and cGvHD were comparable with previously published data in BMT or SCT in CML without Imatinib pre-treatment and with our institution’s experience prior to the introduction of Imatinib. Imatinib prior to allogeneic transplantation does not seem to have a negative impact on the transplantation outcome. Further investigations on larger cohorts are needed in this field.
0164
EARLY OR LATE COMPLETE MOLECULAR REMISSIONS IN CHRONIC MYELOGENOUS LEUKEMIA PATIENTS TREATED WITH IMATINIB RELY ON THE SPEED TO ACHIEVE A COMPLETE CYTOGENETIC REMISSION STATUS AND ON EARLY QUANTITATIVE BCR-ABL LEVELS

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In chronic phase (CP) chronic myelogenous leukemia (CML) treated with imatinib (IM), the majority of patients that are in CCR still harbour, apparently indefinitely, a stable molecular disease, while IM is maintained. A small proportion of patients become RQ-PCR negative (transcripts levels <10-5) and there is still some controversy to know if these patients are cured. We retrospectively analyzed a cohort of 57 patients that became, at least once, BCR-ABL negative in the blood (Taqman® technology, sensitivity threshold 10-5-10-6, exchange of quality control samples between the 2 laboratories involved), to try to identify predictive factors for early (<12 Months, Group I (G1), 19 patients) versus late (>12 Months, Group 2 (G2), 18 patients) complete molecular remissions (CMR), in an univariate and multivariate analysis. Allogeneic transplantated patients were excluded from this study. All patients were in CP at diagnosis. All patients in G1 had a M2 or M3 diagnosis of CML/002/STI571 protocol - 53/76 (70%) pts treated from line with a dose of 400mg/d. We studied 2 different cohorts of pts in CCR: - 67/191 (35%) pts after 48 months and 50 (G2). Some patients have been treated with IFN prior to IM (12/19 (61%) Ph1+ G1), and 1 had a variant [t(2;9;22), Ph1+ G1]. One patient had a masked Ph1 and 1 had a normal karyotype. None of the patients was in CMR at 3 Mo except 2 in G1. CMR appeared after a median interval of 6 Mo in G1 (0-11.2) and 25 Mo in G2 (12-53.7). Univariate analysis did not find any difference for prior treatment by IFN, Sokal score and associations with Cytarabine or pegylated IFN. One patient had a masked Ph1 and 1 had a normal karyotype. None of the patients was in CMR at 3 Mo except 2 in G1. CMR appeared after a median interval of 6 Mo in G1 (0-11.2) and 25 Mo in G2 (12-53.7). Univariate analysis did not find any difference for prior treatment by IFN, Sokal score and associations with Cytarabine or pegylated IFN. Analysis of variance indicated that a low RQ-PCR value at 6 Mo and at 12 Mo was a significant factor for early CMR (p=0.05 for both). Multivariate analysis by logistic regression for Sokal score, prior treatment with IFN, initial IM dose had no influence on the early or late CMR status, whereas the interval between diagnosis and IM onset (p=0.04, HR=1.45 [1.01-2.08]), probably because there was a longer exposure to IFN; and a shorter time to reach a CCR status were significantly associated with early CMR (p=0.05, HR=0.67 [0.46-0.97]). However, the duration of IFN treatment variable was not significant by itself. With a median follow-up since IM onset of 24 (G1) and 43 (G2) Mo, all patients are alive, 16 out of 19 in G1 and 15 out of 18 in G2, in a stable CMR. In conclusion, when IM induces early CCR, and a quick reduction of BCR-ABL transcripts initially, CMR can be obtained within a year, but CMR are still possible after a longer period. However gain in progression free survival remains to be demonstrated.

0165
THE IMPACT OF NON-COMPLIANCE WITH IMATINIB THERAPY ON HEALTH CARE COSTS IN CHRONIC MYELOID LEUKEMIA PATIENTS

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Background. While compliance to drug therapy is vital to receive optimal patient benefits, the costs of delivering adequate medical care for cancer patients remain an important consideration for society and payers. Aims. This study examined the relationship between compliance with imatinib therapy and health care costs for patients with chronic myeloid leukemia (CML). Methods. Claims data from a large US health plan were used to identify imatinib-treated patients from 6/1/01-3/31/04 who had continuous pharmacy and medical benefits in the 3 months prior to and 12 months following initiation of imatinib therapy, and a diagnosis of CML (ICD-9-CM 205.1, 205.10, or 205.11). Compliance was defined by medication possession ratio (MPR=total days imatinib supplied in the first year divided by 365) and patients were stratified into three segments by MPR (<50%, 50-90%, 90-100%). Total health care costs include hospital, office, laboratory testing, emergency room, and pharmacy. Disease-related health care costs to treat CML were also analyzed. Multivariate analyses were used to examine the association between first-year MPR and first-year health care costs, controlling for age, sex, number of other medications used by the patient, the initial starting dose, year of initial imatinib fill, and complications due to underlying disease. Results. Total 876 imatinib-treated patients were identified of whom 415 had at least 15 months of continuous eligibility. Of these, 277 were non-Medicare CML patients. Total health care costs per patient in the first year of therapy in patients with MPR < 50%, 50-90%, and 90-100% were $172777, $54479, and $41591 respectively (p<0.001). Inpatient care was the leading driver of health care costs followed by medication use and ambulatory care. The corresponding numbers for disease-related health care costs were $113905, $35851, and $34964 (p<0.001). Controlling for the variables listed above, the multivariate analyses demonstrated that a 10% increase in MPR was associated with a 4.7% decrease in total health care costs (p=0.032) and 2.4% decrease in disease-related health care costs to treat CML (p=0.057). For example, when MPR was improved from 75% to 85% for a patient, the annual total health care costs for the patient were reduced by $3100 and the annual CML related health care costs went down by $1200. Conclusions. Improved compliance with imatinib therapy is associated with decreased total health care costs and disease-related health care costs. Improving compliance to imatinib therapy may not only optimize clinical outcomes among patients but may also reduce the overall societal burden of health care costs associated with cancer.
Results. We observed a progressive decrease of the amount of BCR/ABL transcript in pts who achieved CR. At 24 months the median reduction in BCR/ABL transcript level was: a 3-log reduction in late CP pts; a 4-log reduction in early CP pts. In the latter group of pts MR was assessed also at 36 months. So we observed that 36 months after the first dose of IM and PEG-IFN pts who were still in CCR had the median value of BCR/ABL transcript of 0.00001% both in BM and PB. Therefore all these pts achieved a CMR (undetectable levels of BCR/ABL transcript level) observed in the 2 groups of pts were not significant. Nevertheless excellent results were obtained in both groups, with a median reduction in BCR/ABL transcript level of at least 5 log. In the pts treated with a combination of IM and PEG-IFN a further reduction of BCR/ABL transcript (about another log) was observed at 36 months of treatment.

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**0167**

**HOMOHARRINGTONINE IS ASSOCIATED WITH A HIGH PROPORTION OF HEMATOLOGIC RESPONSE IN CML PATIENTS WITH HEMATOLOGIC FAILURE TO IMATINIB**

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**Background and Aims.** Imatinib mesylate (IM) is now the gold standard for first line therapy in patients with chronic myelogenous leukemia (CML). Homoharringtonine (HHT) is an alkaloid obtained by an original hemi-synthetic process from Cephalotaxus. HHT having shown activity in vitro as a single agent and as a combination therapy in patients presenting with primary or secondary hematologic resistance. **Methods.** We retrospectively analyzed data from all our patients in chronic phase (CP) or accelerated phase (AP) CML who received HHT as a salvage therapy in order to achieve a complete hematologic response, aiming so to determine its role in the current context of the disease management. **Results.** In total, 15 CML (CP, n=10 and AP, n=5) patients started HHT between 09-2000 and 10-2004 for lack or loss of hematologic response in the two institutions. Main previous therapy was interferon α (IFN, n=6), including 3 patients with IFN + Ara-C, or IM (n=9), including IM 400 mg per day for 8 patients, and IM 600 mg per day for 1 patient, and IM / Ara-C ratio was 10/1 for CP median age at first dose was 60 years (range: 35-74). Median duration of treatment was 49 months (range: 18-121). HHT was administered at 1.25 to 2.5 mg/m² daily dose, as a continuous 24h infusion or as 2 divided doses via the subcutaneous route. The course durations were maximum 6 days when combined with IM (n=5) or Ara-C (n=4), and up to 14 days when used as a single therapy (n=5). Of these 15 patients, 11 (73.3%) achieved a complete hematologic response (CHR) after 2 courses in median (range: 1-6), including 1 patient in accelerated phase and 5 patients with BCR-ABL kinase domain (KD) mutations (E255K, M244V, F317L + K247R, V244Q; Y253H). Responding patients received HHT as a single therapy (n=5), or combined with IM (n=5) or Ara-C (n=5). One additional patient who was in AP returned to chronic phase after 2 HHT + Ara-C courses. There was no hematologic response in the 3 remaining patients. Two of them received HHT as a single therapy and 1 received HHT + Ara-C. Among the patients detected with a BCR-ABL KD point mutation, all CP patients obtained CHR and the mutated patient in accelerated phase (Q252H) did not respond to HHT. Nine patients (60%) experienced grade 4 hematotoxicity, 6 of them presenting anemia requiring blood transfusion (9 occurrences). There was no significant extra-hematologic toxicity reported. **Conclusion.** HHT as a single or a combination therapy may still be of interest for treating CML patients in the tyrosine kinase inhibitors era in case of resistant disease. Indeed, hematological responses to HHT have been obtained in case of BCR-ABL KD mutations. The role of HHT alone or in combination will be refined with well-designed prospective studies.

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**0168**

**ACETYLELME AND PHOSPHOPROTEOME MODIFICATIONS OF IMATINIB SENSITIVE AND RESISTANT CML CELLS AFTER SHORT CHAIN FATTY ACID HISTONE DEACETYLASE INHIBITOR TREATMENT.**

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**Background.** CML patients may become irresponsive to Imatinib because of resistance developed by amplification of the BCR-ABL genomic locus or by point mutations within the kinase domain of BCR-ABL. Innovative dual SRC/ABL kinase inhibitors with higher power alternative native and imatinib-resistant mutants of BCR-ABL give remarkable therapeutic benefits, but at least one mutation is resistant to any kinase inhibitor (T315I). Given these evidences, the investigation of alternative therapeutic agents effective in CML still remains a subject of primary interest. **Aims.** We analysed whether HDACi short chain fatty acids (SCFAs) valproic acid and butyrylacetate were synergistic with imatinib. SCFAs acetylation of non-histone proteins is not well characterized, we thus compared the acetylate and phosphoproteome of CML cells treated and not treated with HDACi, alone and in combination with imatinib, by immunoproteomic techniques. **Methods.** The human CML cell lines K562, LAMA-84 S (Imatinib sensitive) and LAMA-84 R (Imatinib resistant), K562 and -R and -S were grown in the presence of valproic acid at the escalating doses 0.2 mM to 2 mM or in the presence of butyric acid derivative D1 (0.2-1 mM) for 24 and 48 hrs. Apoptosis was monitored by annexin V test and propidium iodide uptake. Bcr-abl mRNA was measured by real time PCR. Bcr-Ab1 protein expression was determined by western blot with specific antibodies. Total cell proteins were separated by 2D electrophoresis (pH 3-11). We used a pan-pan-acetylated and anti-phosphoestrogen antibody for 2D WB, followed by matching with 2D gel and MALDI-TOF mass spectrometry for protein identification. **Results.** Apoptosis was induced time and dose dependent by VPA and D1. Imatinib was synergistic with both HDACi in inducing apoptosis and cell proliferation arrest (WST-1-assay). VPA and D1 were able to induce a significant decrease in the number of copies of Bcr-abl mRNA both in sensitive and in resistant cells. A significant decrease in BCR-ABL protein expression was observed by WB of total cell lysates from CML cells. Twenty two proteins differentially acetylated were identified. At least two chaperone proteins were identified as target of acetylation after VPA and D1 treatment of CML cells, other targets were proteins involved in the synthesis and stability of RNA. Sixteen proteins differentially phosphorylated were identified. For 13 of these proteins the phosphorylation level was not significantly affected by HDACi in resistant cells, while the combination of both Imatinib and HDACi produced a considerable decrease of phosphorylation in both sensitive and resistant cell lines. This category includes: HSP90, HSP70, HOP1 and nucleophosmin. **Summary/Conclusion.** Short chain fatty acids are not the most powerful HDACis, but have been used successfully in clinical trials. Our analysis show significant evidences of their effects on CML cells in terms of induction of apoptosis and arrest of CML cell proliferation. Further experiments were observed on Imatinib expression and modifications on both acetylated and phosphoproteome. This study characterizes proteome modifications provoked by SCFAs and may help to understand the molecular effects of different HDACi in order to improve their use in combination with imatinib or new SRC/ABL inhibitors.

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**0169**

**IMATINIB THERAPY FOR CHRONIC MYELOID LeUKEmIA PATIENTS WHO RELAPSE AFTER ALLOGENEIC STEM CELL TRANSPLANTATION: A MOULAR ANALYSIS**

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**Background.** Allogeneic stem cell transplantation (SCT) is to date the only curative therapy for CML patients, but relapse is still one of the major causes of failure. Discontinuation of the immunosuppressive therapy and donor lymphocyte infusion (DLI) is the treatment of first choice for CML recurrence after allografting, since induces durable remission in a substantial number of patients. Nonetheless, it requires the availability of the donor and may be associated with severe graft-versus-host-
with reconstitution of full donor chimerism without increasing of achieved cytogenetic and molecular responses, which were associated comparable with that expected from treatment with DLI alone. All patients CMR.

was lost in 7 cases and was fully recovered after the achievement of STROHOLM, Sweden

Methods. Patients underwent allogeneic, non T-cell depleted, standard conditioning regimen SCT. At evidence of relapse, 5 patients were in immunosuppressive therapy, which was discontinued. At start of imatinib, five patients were in hematological relapse, Nine had a cytogenetic relapse and two a molecular relapse. Results. Median follow-up is 33 months (range 12-45). All patients achieved a complete cytogenetic response (CCgR) within 12 months. Molecular response was evaluated in bone marrow and/or peripheral blood samples at start of imatinib therapy and every 3 months during treatment by a standardized real-time quantitative PCR (RT-Q-PCR) method and/or by qualitative nested PCR. Complete molecular response (CMR) was defined as reduction of BCR-ABL/B2 Microglobulin below 0.00001 or negativity of qualitative nested PCR in bone marrow samples. Eight patients achieved and maintained a stable CMR. In seven patients, CMR has been achieved but lost at least once during follow-up. In these patients, median duration of longer CMR was 12 months (range: 3-24). All patients are in ongoing treatment with imatinib except for one patient who discontinued the therapy 6 months ago and maintain a CMR. No further treatment was administered in all but two patients, who received DLI after the achievement of CMR. Chimerism analysis has been performed by VNTR analysis or FISH in 8 patients. Prior to imatinib therapy, full donor chimerism was lost in 7 cases and was fully recovered after the achievement of CMR. Conclusions. In our experience, response rate to imatinib is comparable with that expected from treatment with DLI alone. All patients achieved cytogenetic and molecular responses, which were associated with reconstitution of full donor chimerism without increasing of GVHD. Moreover, no major side effects were observed. Although no direct comparison may be made, the data suggest that in our patients percentage of and time to molecular response to imatinib appeared to be better than in newly diagnosed CP CML patients, possibly due to residual GVL effect. An important observation is that even patients relapsed in advanced phase of disease obtained durable molecular responses (median duration of CMR: 20 months, range:6-24). Compared to other therapeutic approaches, our experience confirms that imatinib is effective and feasible, with a very high overall response and a manageable side-effects profile, at least in the short-term.

INFLUENCE OF CYP3A4 ACTIVITY ON IMATINIB RESPONSE IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA

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Background. Imatinib, also known as Gleevec/Glivec, is a potent Bcr-Abl tyrosine kinase inhibitor currently used for the treatment of chronic myeloid leukemia (CML) patients. Imatinib induces complete cytogenetic responses (CCR) in the majority of patients with CML in chronic phase (CP). However, a subgroup of patients is refractory at the cytogenetic level. Imatinib is mainly metabolised via CYP3A4 although several other CYP enzymes have been shown to be involved. The main metabolite CGP74588 a product of CYP3A4 is pharmacologically active, and shows similar potency and selectivity as imatinib. However, the influence of the metabolic pathway on the effect of imatinib and the activity of the metabolites need to be further investigated.

Aims. The aim of this study was to investigate the role of the drug metabolising enzyme i.e. the CYP3A4 activity in vivo, for the response to imatinib treatment. Methods. 16 chronic myeloid leukemia patients were included in the study. To assess the in vivo CYP3A4 activity the patients were given 250 mg of quinine, 16 h later a blood sample was collected and the ratio of quinine/3-hydroxyquinine was measured using HPLC. The response of imatinib treatment was evaluated by cytogenetic analysis and the patients were divided into CCR within nine months and partial cytogenetic responders (PCR) who had failed to achieve a CCR. Results. Patients with CCR showed significantly (Mann-Whitney U-test, p=0.013) higher CYP3A4 activity (low Quinine ratio, mean = 9.4, SD = 2.7) compared to patients that were PCR (high Quinine ratio, mean = 0.5, SD = 0.3), see figure. Conclusions. CML patients with high CYP3A4 activity respond better to imatinib treatment than patients with low activity. Clinically, it would be advantageous to identify such patients a priori, since they may benefit from more aggressive therapy. This also indicates that the effect and potency of the metabolites might be of clinical importance.

0170

IMATINIB 400 MG IN LOW SOKAL RISK CML PATIENTS: EARLY RESULTS OF AN OBSERVATIONAL, MULTICENTRIC TRIAL OF THE GIMEMA CML WP

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Background. Imatinib 400 mg is the established first line treatment of chronic myeloid leukemia (CML) in chronic phase. The efficacy of imatinib in early chronic phase has been demonstrated by multicentric randomized controlled trials like the IRIS trial (O’ Brien et al NEJM 348:11, 2004). Large multicentric studies aimed to evaluate the impact of imatinib 400 mg outside strictly monitored trials are not yet available. Aims. The GIMEMA (Gruppo Italiano Malattie Ematologiche dell’Adulto) CML Working Party opened in January, 2004, an observational study (serial n. CML/023) to investigate the efficacy of imatinib 400 mg in newly diagnosed CML patients. Methods. Clinical and anagaphical data were collected through a web-based system. Responses were evaluated at fixed time-points during treatment. Hematologic: continuously; cytogenetic at 6 and 12 months (local labs); molecular response at 3, 6 and 12 months. Peripheral blood samples for quantitative molecular analysis (RT-Q-PCR, Bcr-Abl/Ab1 × 100 - Taqman) were centralized in Bologna at 3, 6 and 12 months. Patients. Overall, 54 italian centers enrolled 209 (188 evaluable) low Sokal risk patients between January 1, 2004 and November, 2005. Median age was 44 yrs (range 20-69), 117 male and 71 females. 22 patients are evaluable for response at 3 months, 151 at 6 months and 84 at 12 months. The median observation time is 6 months.

Results. At 3 months, 95% of the patients reached a stable complete hematologic response. At 6 months, 81% of the evaluable cases obtained a complete cytogenetic response (100% Ph-neg, CCGR). A major molecular response (MMR) defined as a Bcr-Abl/Ab1 × 100 ratio < 0.1%, was shown in 51% of CCGR patients. At 12 months, the CCGR rate was 88% and the MMR rate in CCGR patients was 57%. At 12 months, 4% of CCGR cases showed a undetectable level of transcript (ratio Bcr-Abl/Ab1 × 100 < 0.00001). With this short observation period, only 1 pt progressed to accelerated/blastic phase, while 2 patients were censored at the time of allogenic stem cells transplantation. SUMMARY AND Conclusions. The preliminary evidences of our observational trial confirm that imatinib 400 mg is a highly effective treatment for CML in early chronic phase, as far the CCGR and MMR response rates. 201 low Sokal risk patients were enrolled in the IRIS trial and received imatinib as first line treatment. The CCGR rate within 12 months was 76% with 66% of patients reaching a MMR (defined as reduction of Bcr-Abl transcript level > 3 logs; control gene Bcr) (T Hughes et al, NEJM 349:15, 2003). Our results (81% and 88% CCGR rate at 6 and 12 months, 51% and 57% MMR at 6 and 12 months) compare favourably with the IRIS trial results.


Figure 1. CYP3A4 activity and Imatinib response.
IMATINIB AND AGING: PRELIMINARY RESULTS OF A SUB-ANALYSIS WITHIN 3 TRIALS OF THE GIMEMA CML WP


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Background. Older age constitutes a poor prognostic variable in Ph+ CML patients, across treatment modalities. Age is included and acts as negative factor in the staging systems most employed (Sokal and Euro). Older patients have been generally excluded from most of the trials employing interferon, due to its toxic effects. Few studies investigated the effect of imatinib in older patients. Cortes et al (Cancer 98, 2003), based on a single centerasic (187 early CP patients overall, 49/187 older than 60 yrs), treated at doses between 400 and 800 mg, showed no difference in response and outcome, observation which needs to be confirmed. Aims. To investigate the effects of age on response and compliance to imatinib. Methods. A sub-analysis within 3 simultaneously running trials of the GIMEMA (Gruppo Italiano Malattie Ematologiche del’Adulto) CML WP (n.CML/021, phase II - ima 800 in intermediate Sokal risk; CML/022, phase III - ima 400 vs 800 mg in high Sokal risk; n. CML/023, observational - ima 400 mg) have been performed. Overall, 404 patients have been enrolled (January 2004 - November 2005). It enrollment 85/404 (21%) were > 65 yrs (median age 71, range 65-85) and 319/404 (79%) < 65 yrs (median age 46, range 18-84). Sokal risk distribution was different between the 2 groups: low Sokal risk cases were 15% in older cohort vs 54% in younger cohort (p<0.01). 21% of older and 22% of younger pts received high dose (800 mg) of imatinib front-line. Timing of response evaluation: hematologic, continuously; cytogenetic, at 6 and 12 months; molecular, at 3, 6 and 12 months.Pt samples for quantitative analysis (RT-Q-PCR, Bcr-Abl/Abl x 100 - Taqman) were centralized in Bologna at 3, 6 and 12 months. Results. The numbers (%) of evaluable cases (older/younger) at 3, 6 and 12 months were: 85/519 (100/100%), 59/251 (69/79%) and 27/141 (33/33%). At 3 months both groups achieved a 95% complete hematological response (CHR) rate. At 6 months, the complete cytogenetic response (CCgR) rates were (older/younger) 66%/80% (p=0.39). The major molecular response (MMR, defined as a Bcr-Abl/Abl x 100 ratio < 0.1%) rates (CCgR only) were 67%/49% (p=0.08). At 12 months, CCgR rates were 81%/68% (p=0.67) and MMR 50%/60% (p=0.04). With a median observation time of 6 months, 1 pt (1%) of older cohort and 4 (1%) of younger cohort progressed to accelerated/blastic phase. Summary and Conclusions. This sub-analysis was generated from 3 trials with different aims and dosages of imatinib. The observation period is still short. However, it is noteworthy that notwithstanding a worse risk distribution of older cases (15% low risk vs 54% for younger), results at 6 and 12 months are comparable. The only significant difference was demonstrated for MMR at 12 months. Consequently, we may foresee that the long-term survival and progression free survival will not differ between the 2 groups. Acknowledgments. Supported by: COFIN 2003, FIRB 2003, A.I.R.C, C.N.R., Fondazione del Monte di Bologna e Ravenna, LeukemiaNet, A.I.L.

BCR-ABL REDUCES CCN3 EXPRESSION THEREBY EVADING NEGATIVE GROWTH REGULATION

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Background. Chronic Myeloid Leukemia (CML) is characterized by expression of the constitutively active BCR-ABL tyrosine kinase. Consequently, we have identified downregulation of the negative growth regulator, CCN3, as a result of BCR-ABL kinase activity and detected reduced CCN3 expression in human CML cell lines and primary human CML cells. Aims. To identify the relationship between BCR-ABL and CCN3 expression and the functional consequence of expressing CCN3 in BCR-ABL+ cells. Methods. Real-time PCR was used to examine the relationship between BCR-ABL and CCN3 expression in human K562 cells using siRNA directed against BCR-ABL or the BCR-ABL tyrosine kinase inhibitor, Imatinib. CCN3 function was investigated in K562 cells transfectd with vector or vector containing CCN3 construct by assessing cell growth using flow cytometry and colony formation on methylcellulose. Results. Parental K562 cells showed high expression of BCR-ABL whilst CCN3 expression was present at low levels. Treatment with siRNA directed against BCR-ABL resulted in a 3.7 fold decrease in BCR-ABL and 6.1 fold increase in CCN3 expression (mean Ct change 1.9±0.2 and 2.6±0.5 for BCR-ABL and CCN3 respectively; n=3, p=0.001). Similarly, cells treated with imatinib, a known BCR-ABL inhibitor, displayed a 5.9 fold decrease in BCR-ABL expression and a 4.2 fold increase in CCN3 expression (mean Ct change 2.5±0.1 and 2.1±0.2 for BCR-ABL and CCN3 respectively; n=3, p=0.001). To investigate CCN3 function, we expressed CCN3 in BCR-ABL expressing cells. K562 cells were transfected with either the pcB6+ vector or pcB6+ vector containing the CCN3 construct. Cell cycle analysis was performed: CCN3 expression in pcB6+ cells resulted in an accumulation of cells in the subG0 phase of cell cycle, indicative of cell death (mean for subG0 9.9%±4.6 and 21.3%±0.7 for the pcB6+ vector alone and pcB6+ vector containing CCN3 construct respectively). In addition, CCN3 expression reduced the clonogenic capacity of BCR-ABL+ cells. K562 cells transfected with vector containing CCN3 construct formed significantly fewer colonies on methyl cellulose in comparison to cells that had been transfected with the pcB6+ vector alone (n=3, p=0.027). Conclusions. This study demonstrates a reciprocal relationship between CCN3 and BCR-ABL expression. CCN3 is known to be a down-regulator and increased expression of CCN3 in BCR-ABL+ cells inhibits proliferation and decreases clonogenic potential. Thus CCN3 down-regulation mediated by BCR-ABL offers growth advantage to hematopoietic cells.

THE PERSISTENCE OF P190 BCR-ABL TRANSCRIPTS IS ASSOCIATED WITH LOWER PROBABILITY OF MOLECULAR RESPONSE TO IMATINIB IN EARLY AND LATE CHRONIC PHASE CML PATIENTS.


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Background. It has been demonstrated that the about 70% of p210CML patients in chronic phase (CP) at diagnosis co-expressed p190 BCR-ABL transcripts, although at a much lower level. In this study we have assayed by real-time quantitative reverse transcriptation PCR (qRT-PCR) for the co-expression of p190 and p210 BCR/ABL transcripts 1) at diagnosis in previously untreated CP-CML patients, and 2) during the treatment with imatinib in 2 different groups, those with previously untreated disease (early CP-CML) and those who previously failed IFN therapy (late CP-CML). The clinical relevance of p190 BCR-ABL monitoring in CML pts under Imatinib is still unknown. Materials and Methods. Bone marrow (BM) samples were obtained from 83 pts with CP-CML treated with Imatinib at a conventional oral dose. These included 192 samples from 40 pts with late CP-CML and 140 samples from 40 pts with early CP-CML. Median follow-up was 2.6±0.5 for BCR-ABL and CCN3 respectively. In addition, CCN3 expression reduced the clonogenic potential of BCR-ABL+ cells. K562 cells transfected with vector containing CCN3 construct formed significantly fewer colonies on methyl cellulose in comparison to cells that had been transfected with the pcB6+ vector alone (n=3, p=0.027). Conclusions. This study demonstrates a reciprocal relationship between CCN3 and BCR-ABL expression. CCN3 is known to be a down-regulator and increased expression of CCN3 in BCR-ABL+ cells inhibits proliferation and decreases clonogenic potential. Thus CCN3 down-regulation mediated by BCR-ABL offers growth advantage to hematopoietic cells.

The persistence of p190 BCR-ABL transcripts is associated with lower probability of molecular response to imatinib in early and late chronic phase CML patients.

Conclusions. To test if the persistence of p190 transcripts during the follow-up was predictive of MMR, we divided CML pts in two groups, those with 0 or 1 p190 BCR-ABL+ samples (group 0-1) and those with 2 or more positive samples (group ≥2). We found that for late CP-CML, real-time PCR was showed a significant lower probability to obtain MMR compared to group 0-1 [17/24 (71%) vs 5/19 (26%) p=0.0039]. The same result was observed for early CP-CML [15/21 (71%) vs 6/18 (33%) p=0.017]. In conclusion, we confirm the frequent co-
expression of p190 and p210 transcripts by CP-CML patients at diagnosis. However, some patients with low levels of p210 transcripts continue to display p190 expression. The persistence of p190 signals despite the 2-3-log fall in p210 BCR-ABL levels may be of prognostic value. The significance of the lack of correlation between p190 and p210 transcript levels warrants further investigations and may disclose unfolded biological relevance.

0175 IMPACT OF BCR/ABL GENE EXPRESSION ON THE PROLIFERATIVE RATE OF DIFFERENT SUBPOPULATIONS OF HEMATOPOIETIC CELLS IN CHRONIC MYELOID LEUKEMIA


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Background and Aims. Despite the effects of BCR/ABL on cell proliferation, no study has been reported so far in which the proliferative rate of different hematopoietic cell compartments from chronic myeloid leukemia (CML) has been compared to normal bone marrow (NBM). In order to gain further insight into the potential impact of BCR/ABL gene expression on leukemic CML cells, we compared the proliferative rate of different BM cell subpopulations from CML patients and normal subjects and explored the correlation between the proliferation of each cell population from CML with BCR/ABL gene expression in highly-purified fractions of BM cells. Methods. A total of 26 BM samples corresponding to 15 patients diagnosed of CML and 11 NBM were studied. The proportion of S+G2/M cells was analyzed on CD45/CD19/DRAQ5, CD34/CD117/DRAQ5, CD11b/CD13/DRAQ5, CD36/CD14/DRAQ5 cell population from CML with BCR/ABL gene expression in highly-purified fractions of BM cells. Fluorescence activated cell sorting (FACS) of BM cell populations from CML patients (n=7) was performed using a FACSAna flow cytometer (BDB) and the FACS DiVa software (BDB). A CD38/CD34/CD19/CD13/CD11b/CD45 6-colour staining was used to sort CD38+/CD34+/CD19 myeloid hematopoietic stem and precursor cells, CD34+ myeloblasts (SSChigh/CD45low/CD11b−/CD13− non-autofluorescent cells), promyelocytes (SSChigh/CD45low/CD11b+CD13− non-autofluorescent events), myelocytes/meetamyelocytes (SSChigh/CD45−/CD11b+CD13− non-autofluorescent events) and bands/neutrophils (SSC−/CD45−/CD11bhigh/CD13−/CD13+ non-autofluorescent events). Quantitative-real time PCR (qPCR) analyses were performed on whole BM samples and purified cell populations in MicroAmp 96-well optical plates on an ABI PRISM 7700 sequence detection system (PE Applied BioSystems, Foster City, CA) and the results expressed as normalized BCR/ABL copy numbers (NCN) per one copy of the GUS gene x10. Conclusions. These results suggest that in CML, BCR/ABL expression is associated with an increased proliferation of CD34+ myeloid HPC but not of other more mature myeloid precursors as also confirmed by the lack of direct correlation between the amount of BCR/ABL transcripts and the proportion of S+G2/M-phase cells.

0176 SURVIVIN AND CIAP-1 GENE EXPRESSION IS LINKED TO CHRONIC MYELOID LEUKEMIA PROGRESSION AND POOR RESPONSE TO IMATINIB


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Background. Chronic myeloid leukemia (CML) is a myeloproliferative disease in which bcr-abl oncogene enhances survival of leukemic cells through modulation of proapoptotic and antiaptiopotic molecules. The IAPs (inhibitor of apoptosis proteins) are a family of caspase inhibitors that block the execution phase of apoptosis. Overexpression of IAPs confers chemoresistance and, in some groups of cancer patients, is associated with a poor prognosis. Fully understanding the basic apoptotic pathway and its regulation in Bcr-Abi-positive cells will unveil more targets for manipulation, which can be translated into novel therapies. Aim. The objective of this work was to determine the IAPs gene expression (ciap-1, ciap-2 and survivin) in 15 healthy individuals and on 71 CML patients (20 chronic phase, 15 accelerated phase, nine blastic phase, 20 in cytogenetic remission and seven refractory patients post-imatinib). Results. The results are expressed by relative expression e.g. ratio of investigated gene to the reference GAPDH gene. survivin and ciap-1 gene overexpression was observed in 61 (86%, p<0.001) and 55 (77%, p<0.001) patients, respectively. The survivin levels (mean/SD) were: 0.11/0.05 in controls; 0.53/0.14 in chronic (CP), 2.1/0.55 in accelerated (AP), 9.6/3.3 in blastic (BP) phases, 0.04/0.02 in CML remission and 18.08/8.0 Gleevec-refractory patients. The ciap-1 expression was 17.6/3.53 in controls; 31.02/5.9 in CP, 15.75/2.6 in AP, 75.27/15.49 in BP, 32.8/5 in CML remission and 59.04/12.05 in Gleevec-refractory patients. Conclusion. Therefore, there was an association between survivin (p<0.001) and ciap-1 (p<0.001) mRNA level with CML stage and response to imatinib. The ciap-2 gene expression observed in healthy controls and CML patients is similar (p>0.05). Taken together our results suggest that each IAP homologue has a different mechanism of action and because more than one member of this family may be overexpressed in CML, successful treatment strategies for this disease will be defined by the ability to block all of the IAP expressed or to associate its inhibitor with other therapies. Supported by: CNPq, FAPESP, Instituto de Investigação em Imunologia-Instituto do Milênio/CNPq e IIEP-HIAE

0177 ABL KINASE DOMAIN MUTATIONS ARE IMPORTANT MECHANISMS OF RESISTANCE IN ASIAN PATIENTS WITH IMATINIB-RESISTANT CHRONIC MYELOID LEUKAEMIA


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Introduction. While the efficacy of imatinib in chronic myeloid leukemia (CML) is without doubt, resistance remains a problem, especially in the advanced phases. The most common mechanism of resistance is point mutations leading to amino acid substitutions in the Abl kinase domain and has been described mainly in the Western population. Little information is currently available if this mechanism is also common among Asian patients. Methods. Samples from 32 patients with suboptimal responses or progression on imatinib have been analysed so far. RNA was extracted from peripheral blood and complementary DNA synthesised using random hexamers. The Abl kinase domain was amplified using a two-step semi-nested reverse transcriptase/polymerase chain reaction (RT/PCR) and the PCR products subjected to direct sequencing. Results. The median age was 47 (19-77) years with a equal sex distribution. There were 15 Chinese, 9 Indian and 8 Malay patients. Thirteen patients were in the chronic phase (CP), 6 in accelerated phase (AP) and 3 in blast phase (BP). The median duration of imatinib treatment was 27 (7-65) months. A total of 20 mutations were detected in 14 patients, with 8 located in the ATP-binding loop, 4 in the imatinib-binding site, 2 in the catalytic domain and 2 in the activation loop. The remaining 4 were in other sites within the Abl kinase of type I mutations. This new mutation will be verified by amplifying the ABL alleles to exclude polymorphisms. Four patients had more than one mutation. Of these 14 patients, 12 were enrolled into clinical trials with second generation Abl
kinase inhibitors (dasatinib, Bristol-Myers Squibb or AMN107, Novartis). Mutation testing was also performed in 6 patients after 3 months of dasatinib (see Table).

<table>
<thead>
<tr>
<th>Patient</th>
<th>Phase</th>
<th>Mutation pre-dasatinib</th>
<th>3 months post-dasatinib</th>
<th>Response to dasatinib at 3 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AP</td>
<td>F359V</td>
<td>T315I</td>
<td>No cytogenetic response</td>
</tr>
<tr>
<td>2</td>
<td>AP</td>
<td>T240A</td>
<td>G256E</td>
<td>Complete haem response</td>
</tr>
<tr>
<td>3</td>
<td>AP</td>
<td>H369R</td>
<td>E255K</td>
<td>Complete haem response</td>
</tr>
<tr>
<td>4</td>
<td>CP</td>
<td>E355G</td>
<td>T315I</td>
<td>Minor cytogenetic response</td>
</tr>
<tr>
<td>5</td>
<td>CP</td>
<td>E255V</td>
<td>T315I</td>
<td>Minor cytogenetic response</td>
</tr>
</tbody>
</table>

Haematologic progression was observed with the development of the T315I mutant in 2 patients and in 1 patient, a minor cytogenetic response was associated with a relative reduction in size of the mutant clone. Conclusions. Our study shows that Abl kinase mutations are common in Asian CML patients resistant to imatinib. Currently, mutation testing is only available in a few laboratories across the continent. It is therefore important that screening for mutations be performed routinely in imatinib-resistant Asian CML patients as this will have an impact on therapeutic decision making.

0178 INCREASED ANGIOGENESIS IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA: CLINICAL AND MORPHOLOGICAL ANALYSIS

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The degree of intratumoral neo-vascularization as well as the expression of some proangiogenic factors is of prognostic significance in some solid tumors. It is still to be elucidated the possible prognostic role of increased angiogenesis in patients with haematological diseases. The aims of the present study are: (1) to analyze the neo-vascularization of bone marrow in patients with chronic myeloid leukemia (CML); (2) an assessment of bone marrow cellular expression of Vascular endothelial growth factor (VEGF) and its receptor VEGFR-2 (KDR) as well as the plasma levels of VEGF in CML; (3) to analyze the correlation dependency of angiogenic factors with the prognostic and biologic markers of the disease. A totally of 87 patients with CML as well as 80 healthy individuals were analyzed for VEGF plasma levels by using ELISA technique. Immunohistochemical methods were applied to visualize the vascular structures as well as the VEGF/KDR cellular expression in 17 trephine biopsies from newly diagnosed patients with CML and in 15 normal bone marrows. We observed that the mean vessel count per field was 3.13 per 0.0625 mm² in normal bone marrows vs. 24.6 per 0.0625 mm² in CML (p<0.001).

Figure 1. Increased MVD in CML bone marrow (A) comparing to the normal (B) case. (Von WF immunostaining)

The VEGF/KDR cellular expression levels were nearly 5-fold that in normal control samples and the VEGF plasma levels were significantly higher in CML group (Mean 429 vs. 36.8 pg/ml; p<0.001). A good correlation was found between plasma VEGF and platelets as well as leucocytes but not with the blast per cent and Hasford prognostic score. Likewise, plasma VEGF levels could not predict the acceleration of the disease, moreover, their levels decline with the progression of CML. We found a good correlation between MVD and cellular VEGF/KDR expression but only cellular KDR is in a significant correlation with Hasford prognostic score. According to our results the high MVD, VEGF and KDR expressions indicate that angiogenesis is an inevitable event in the pathophysiology of CML. The complex angiogenic assessment of bone marrow provides more reliable information about the occurrence and the significance of this process in CML than using VEGF plasma concentration alone. That is to say, the precisely defined patients with CML, which have a high rate of angiogenic activity in bone marrow, could benefit by angio-suppressive therapy.
Non-Hodgkin’s Lymphoma - Clinical I

A PHASE II, MULTICENTER, SINGLE-AGENT STUDY OF BENDAMUSTINE HCl IN PATIENTS WITH RITUXIMAB-REFRACTORY INDOLENT B-CELL NON-HODGKIN’S LYMPHOMA

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Background. Bendamustine HCl (TREANDA™) is a novel, multifunctional, hybrid, cytotoxic agent with novel mechanisms of action. Unlike other commonly used chemotherapeutic agents, bendamustine induces durable DNA damage resulting in rapid cell death in apoptosis-resistant cancer cell lines through the apoptosis-independent pathway of mitotic catastrophe. Studies have reported single-agent activity in patients with relapsed/refractory non-Hodgkin’s lymphoma (NHL), chronic lymphocytic leukemia, multiple myeloma, and breast cancer. Aim. This study evaluated the efficacy and safety of bendamustine in patients with NHL who had relapsed and were refractory to prior rituximab treatment. Methods. This Phase II, multicenter study enrolled patients with relapsed, indolent, or transformed, rituximab-refractory B-cell NHL. Rituximab-refractory disease was defined as no response or progression within 6 months of completing rituximab treatment. Patients received bendamustine 120 mg/m² IV over 30 to 60 minutes on days 1 and 2 every 21 days for 6 cycles. Response was measured using the International Working Group criteria. Results. The intent-to-treat (ITT) population consisted of 77 heavily pretreated patients (53% male) with a median of 4 prior systemic therapies (range: 1-9), enrolled in 14 sites in the US and Canada. Median age of patients was 63 years (range: 38-84), and 87% had stage IV (bone marrow involvement). 11% had peripheral blood involvement, 11% had bulky disease, 15% B symptoms, 6% ECOG score ≥2. 23% had hemoglobin <12 g/dL. LDH was above normal in 15% and b2-microglobulin in 45%. 11% had an autoimmune Background. HCV serology was positive in 24% (9/38). With the IPI score 87% ranked in the low risk, 22% in the low-intermediate, 35% in the intermediate-high, and 7% in the high risk category. Using the FLIPI score, 33% were classified as low risk, 34% as intermediate risk, and 33% as high risk. After treatment, 57% achieved a complete response and 24% a partial response, for an overall response rate of 81%. At a median follow-up of 2.6 years, no patient developed splenic or MALT involvement. 5-years and 10-years OS is 69% (95% CI 52-86%).

Figure 1. OS of primary nodal MZL according to FLIPI.

Death occurred in 10 pts (related to NHL in 9, to another neoplasm in one). In univariate analysis the following factors were associated with shorter event-free survival (EFS): B symptoms (p=0.001), high vs intermediate-med low risk FLIPI score (p=0.009). The following factors were associated with worse overall survival: high vs intermediate vs low risk FLIPI score (p=0.02) (Figure 1), age > 60 years (p=0.05), LDH above normal (p=0.05). HCV positivity was of borderline significance (p=0.06). In multivariate analysis hemoglobin < 12 g/dL (p=0.02, HR 14.8) was predictive of shorter EFS. Concerning overall survival, only the FLIPI retained statistical significance in predicting a worse outcome (p=0.02, HR 3.5). Positive HCV serology was of borderline significance (p=0.06, HR 4.4). Conclusions. Among marginal zone neoplasms, primary nodal marginal zone lymphoma appears a distinct disorder with an indolent behaviour. The association with HCV infection (25%) is particularly high in comparison with non-marginal zone lymphomas. Considering the prognostic assessment of this rare disease, the FLIPI score is effective in detecting patients at worse prognosis with the same power as in
folicular lymphoma. Thus, the application of the FLIPI may be of clinical value for treatment decision also in primary nodal marginal zone lymphoma.

**0181**
PRELIMINARY EVALUATION OF EFFICACY AND TOXICITY OF TWO DOSES SCHEDULES OF BORTEZOMIB PLUS R-CHOP REGIMEN IN FRONT-LINE B LYMPHOMA PATIENTS

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Background. Bortezomib (Velcade®) is the first of its class of proteasome inhibitors tested in humans that showed promising activity as single agent in several tumor types, and especially in hematologic malignancies, in phase II studies. Only few reports have been made with combination chemotherapy. **Aims.** In March 2004, we initiated a phase II study with the bortezomib and R-CHOP regimen to evaluate efficacy and toxicity of the combination in NHL B patients. **Methods.** All patients received 6 cycles of standard Rituximab-CHOP (day 21= day1). Patients were randomized between two doses schedules of administration of bortezomib: A (bi-weekly: day 1,4,8 and 11), B (weekly: day 1 and 8). For the first 24 patients (step 1), Velcade was administered at 1mg/m² in group A and 1.8 mg/m² in group B. For the next 24 patients (step 2), in absence of severe toxicity in step 1, it was planned to increase velcade at 1.3 mg/m² in group A and 1.6 mg/m² in group B. **Results.** We report here the safety results on 29 patients, there were 11 females, 18 males, with a median age of 59 years old (32-76). Histology: 2 Lymphoplasmocytoid Lymphoma, 5 Marginal Zone Lymphoma, 8 Follicular lymphoma; 3 Follicular Lymphoma with histological Transformation; 3 Mantle Cell Lymphoma and 8 Diffuse Large B Cell Lymphoma without adverse factor (IPI=0). Performance status ≥2: 0 ; LDH > N : 9 ; number of extra-nodal sites > 1 : 11. 27 patients received 6 cycles (1 patient was in progression after 5 cycles and 1 patient did not receive the 6th cycle). In step 1, group A: 90 to 100% of scheduled dose of bortezomib was administered. For group B it was 99 to 100%. In step 2, group A, 78 to 100% of scheduled dose of bortezomib was administered. It was 100% for group B. Dose reduction were made after cycle 4. G-CSF and EPO support was used when necessary. Grade 3-4 hematotoxicity (per cycle) occurred in 13% for platelets, 45% for leukocytes. There was no red blood cells transfusion. The neurological grade 3-4 toxicity occurred in 5 patients. All occurred in the biweekly group (step 1 n=2/12; step 2 n=3/5) and none in the weekly group. The observed major toxicities grade 3-4 were nausea 1/29, diarrhoea 1/29, 1 serious infections and 1 angina pectoris. After 6 cycles, the overall response rate was 97% (28/29) with 26 patients in CR/CRU (>90%).

**0182**
CLINICAL FEATURES AND TREATMENT OUTCOMES OF ANGIOIMMUNOBLASTIC T-CELL LYMPHOMA

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Angioimmunoblastic T-cell lymphoma (AITL) is a rare subtype of lymphoma, making up only 1% to 2% of non-Hodgkin’s lymphomas. The objective of this study was to investigate clinical features and treatment outcomes in patients with AITL. From September 1990 to June 2005, 65 patients diagnosed as AITL were included in the analysis. About one half of patients presented with poor performance status (ECOG > or =2); 72.3% of patients were categorized as high intermediate or high-risk group according to IPI, and most of patients (95.4%) were diagnosed at advanced stage. At diagnosis, 27 patients (41.5%) presented with malignant pleural effusion; 22 patients (33.8%) had a skin involvement. The median overall survival for all patients with AITL was 15.1 months (95% CI, 6.7-23.5). The response rate of 50 patients who had been treated with primary anthracycline-based chemotherapy was 80.0%; 62.0% CR rate, 18.0% PR rate. After a median follow-up of 39.0 months (range 2.4-177.8) in 53 patients who had achieved a CR, 15 patients (45.5%) developed disease recurrence with median CR duration of 45.6 months (95% CI, 25.5-65.7). The median progression free survival of all patients was 7.1 months (95% CI, 2.8-11.4). High dose chemotherapy followed by autologous stem cell transplantations were conducted in 4 patients for salvage therapy, which resulted showed 3 CR, 1 treatment-related mortality. The adverse prognostic factor for survival was only high IPI score in multivariate analysis. In conclusion, although AITLs showed a better response to the conventional anthracycline-based chemotherapy than the others, its response durations were short, therefore the chemotherapy regimen for AITL should be modified or intensified like a high dose chemotherapy followed by autologous stem cell transplantation especially in responsive patients.

**0183**
AUTOIMMUNE DISEASES IN PATIENTS WITH MALT-LYMPHOMA: CHARACTERISTICS AND CLINICAL COURSE

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Background. The development of a Non-Hodgkin's lymphoma (NHL) is one of the most serious complications in patients with autoimmune diseases (AD). Most of these lymphomas originate from B-cells and the extranodal mucosa associated lymphoid tissue (MALT) lymphoma is the most common subtype. Currently, it is unclear whether these lymphomas have a different clinical course or a different responsiveness to therapy compared to their counterparts in patients without autoimmune diseases. **Aims.** We have investigated the clinical characteristics of MALT lymphoma patients and the influence of AD on the clinical course as compared to controls without an underlying autoimmune condition. **Patients and Methods.** We evaluated retrospectively 219 patients with histologically verified MALT lymphoma within a case-control study. In 134 cases, clinical and serologic data to judge the presence of AD were available. The epidemiologic (age), genetic (trisomy 3, trisomy 18, t(11;18), t(14;18) involving IGH/MALT1) and clinical data (site and extent of disease, relapse rate, time to relapse, monoclonal gammopathy) of these patients with autoimmune diseases were compared to those without AD. **Results.** In total, 65/134 patients (47%) suffered from a concurrent AD, with patients being significantly younger (59 vs 67 years, p=0.002). Elevated autoimmune parameters without clinical significance and symptoms were found in 15.7% of patients. Patients with AD had significantly more extragastrectic lymphoma (p=0.011), but showed a comparable number of multifocal disease (p=0.7). Surprisingly, equal relapse rates (p=0.3) and a similar time to relapse were found in both groups (p=0.8, Figure 1).

Figure 1. Time to relapse.

There was no difference with regard to genetic aberrations: trisomy
3 (p=0.057), trisomy 18 (p=0.8), t(11;18) (p=0.1) and t(14;18) (p=0.6). The presence of a monoclonal gammopathy/paraprotein production was evenly distributed (p=0.1) between both groups. Summary/Conclusions. This is the first study to suggest that the clinical course and the genetic aberrations of MALT lymphoma patients are not related to the presence or absence of autoimmune diseases. However, patients with autoimmune diseases develop MALT lymphomas at a significantly younger age. It was also shown that elevated autoimmune parameters were in fact associated with underlying AD in 85% of cases and were not merely a paraneoplastic phenomenon. Since a significant number of patients with MALT lymphoma suffer from an underlying AD, it is reasonable to determine autoimmune parameters on a routine basis in such patients and to search for the presence of an underlying AD.

**0184**

RITUXIMAB-M/VACOP-B COMBINED WITH RADIOTHERAPY IN PRIMARY MEDIASTINAL LARGE B CELL LYMPHOMA: A PROSPECTIVE ITALIAN INTERGROUP PHASE II STUDY

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Background. Weekly third generation regimens such as MACOP-B or VACOP-B (M/VACOP-B) in combination with involved-field radiotherapy (IFRT) seem to improve lymphoma-free survival of PMLBCL (Zinzani 2002, Todeschini 2004, Savage 2005). The superiority of R-CHOP over CHOP or CHOP-like regimens has been recently demonstrated in younger low risk NHL (Pfreundschuh 2005). Aims. To evaluate the effectiveness and safety of Rituximab added to the standard M/VACOP-B regimens (R-M/VACOP-B) ±IFRT in PMLBCL. Patients and Methods. A total of 40 patients with PMLBCL were treated in six participating centers between February 2002 and July 2005. The median age was 38 years (range 17-54); 21/19 (55%) were females, 50 patients had stage II and 10 stage IV, 38 (95%) presented a bulky disease; LDH was increased in 26 (65%) and 21/33) had a superior vena cava syndrome. According to the age-adjusted IPI score, 24 patients had an IPI = 0-1 and 16 an IPI = 2-3. All patients were treated with standard MACOP-B (30 patients) or VACOP-B (10 patients) regimens plus six cycles of Rituximab (375mg/m²) given at weeks 3, 5, 7, 9, 11, 13. Twenty-six patients (65%) received mediastinal IFRT at a median dose of 36 Gy. The response was evaluated in all patients after six cycles of chemo-immunotherapy, at the end of planned chemotherapy and after IFRT. Results. The response rate after six cycles of the planned R-M/VACOP-B regimen was CR/CRu = 20/30(50%), PR=19(47%) and NR=1(3%). Eight/40 patients received a second line therapy followed by HDT-ASCT (6/8 patients) because considered low responders (PR=7 and NR=1). At the end of the chemoinmunotherapy program 28 patients witnessed a CR/CRu (70%) and 12 a PR (30%). Seven/12 PR patients obtained a CR/CRu following IFRT and 12/26 (46%) patients received HDT-ASCT (6/8 patients) after conventional low responders (PR=7 and NR=1). At the end of the chemoimmunotherapy program 28 patients witnessed a CR/CRu (70%) and 12 a PR (30%). Seven/12 PR patients obtained a CR/CRu following IFRT and 12/26 (46%) patients received HDT-ASCT (6/8 patients) because considered low responders (PR=7 and NR=1). Eight/40 patients received an extended chemotherapy followed by HDT-ASCT (6/8 patients) because considered low responders (PR=7 and NR=1). Eight/40 patients received an extended chemotherapy followed by HDT-ASCT (6/8 patients) because considered low responders (PR=7 and NR=1). Eight/40 patients received an extended chemotherapy followed by HDT-ASCT (6/8 patients) because considered low responders (PR=7 and NR=1).

Conclusions. R-M/VACOP-B are an active therapeutic regimens devoid of severe toxicity for the management of patients with PMLBCL. Further studies are required to demonstrate if the addition of Rituximab to front-line third generation regimens might overcome the need of more aggressive strategies, such as consolidation with IFRT or HDT-ASCT.

**0185**

ASSOCIATION OF REDUCED DOSE INTENSITY AND SURVIVAL IN LYMPHOMA PATIENTS RECEIVING CHOP-21 CHEMOTHERAPY

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Background. Chemotherapy delivery in patients with non-Hodgkin lymphoma (NHL) is sometimes impaired by treatment side-effects. It remains unclear whether moderate reductions in relative (i.e., administered compared to planned) chemotherapy dose intensity (RDI) affect overall survival (OS) and progression-free survival (PFS). To assess the relationship of reduced RDI and overall survival in NHL patients receiving CHOP chemotherapy with a cycle length of 21 days (CHOP-21). Methods. Retrospective audits of NHL patients were conducted in Belgium (lymphodose ‘02) and the UK (Audit of Lymphoma Patients). Variables available from both datasets were merged into a dataset of individual observations, and definitions were harmonised, to allow for comparisons and combined analyses. RDI was averaged across anti-malignant drugs. Potential predictors of survival were assessed using extended Cox proportional hazards regression.

Results. The Belgian study included 211 NHL patients receiving CHOP-21 and the UK study included 78. Of these, 59% (Belgium) vs. 46% (UK) were female. Mean age (SD) at chemotherapy initiation was 63 (14) years (Belgium) vs. 61 (15) years (UK). Ann Arbor stages were distributed 1 25%, II 34%, III 17%, IV 27% (Belgium) vs. I 19%, II 30%, III 27%, IV 24% (UK). During a mean observation time (SD) of 80 (17) months (Belgium) vs. 72 (38) months (UK), 51% vs. 35% of patients died. The Kaplan-Meier survivor functions differed (borderline p=0.06) and the proportion of survivors at 60 months was estimated to be 61% in Belgium vs. 67% in the UK. Mean RDI (SD) was 90% (6%) in Belgium vs. 94% (9%) in the UK (p=0.03). This difference was associated with a higher proportion of Belgian patients experiencing dose delays 27 days (38%) vs. 29% in the UK (p=0.14). The proportion of patients receiving RDI ≤50%, RDI 50-80% and RDI ≥80% was 30%, 25% and 16% (Belgium) vs. 23%, 17% and 7% (UK). Kaplan-Meier plots showed reduced survival for those with reduced RDI, and the effect was strongest when the 90% cut-off (graph), or the 85% cut-off was used. Extended Cox regression using the combined dataset showed reduced survival to be associated with age (hazard ratio 1.03 per year of age, 95% CI 1.01-1.05), RDI ≤50% (hazard ratio 1.8, CI 1.1-2.8), and stage of disease (hazard ratio 2.0 at treatment initiation, CI 1.5-2.9). The strength of the association with stage of disease decreased over time. Summary/Conclusions. This analysis confirms earlier reports that reduced
Background. The rapid adoption of FDG PET/CT scanning for imaging lymphoma has occurred in the absence of a large body of experience of this imaging modality. Weekly bortezomib is active in animal models for myeloma and is expected to be more convenient than the approved twice-weekly regimen. Weekly dosing was therefore studied in NHL. Aims. This randomised, phase 2 study investigated the response rate to bortezomib plus rituximab, weekly or twice-weekly, in patients with relapsed follicular lymphoma (FL) or marginal zone lymphoma (MZL). Methods. Eligibility criteria included CD20+ FL or MZL with measurable disease, and Karpovsky Performance Status ≥50% (ECOG 0–2). No prior bortezomib was allowed and patients who had received a prior regimen of rituximab had to have responded and have a TTP of ≥24 months. Patients received bortezomib 1.3 mg/m² twice-weekly on days 1, 4, 8 and 11 of a 21-day cycle (Arm A) or bortezomib 1.6 mg/m² weekly on days 1, 8, 15 and 22 of a 33-day cycle (Arm B) for up to 15 weeks (5 and 3 cycles in arms A and B, respectively). Starting from day 1, rituximab 375 mg/m² was administered weekly for 4 weeks in both arms. Response was evaluated by International Workshop Criteria. Results. 81 patients were enrolled between April 2004 and August 2005, and 74 (35 Arm A, 39 Arm B) were evaluable for response. Of these 81 patients, the majority had FL (80% Arm A, 93% Arm B); 76% of patients in Arm A received prior rituximab compared with 82% of patients in Arm B. 11 treated patients (27%) in Arm A and 9 (18%) in Arm B completed all planned cycles. Median bortezomib dose received was 15.9 mg/m² (61% of the maximum expected) in Arm A, and 18.9 mg/m² (98% of the maximum expected) in Arm B. Overall response rate was 51% (2 CR + 2 CRu + 14 PR) in Arm A, and 54% (2 CR + 3 CRu + 16 PR) in Arm B. 11 patients had progressive disease (6 Arm A, 4 Arm B, 1 patient died (Arm A)). Response was similar in patients who previously received rituximab compared with the total study population. Median progression-free survival has not yet been reached (median follow-up 3.9 months). Treatment was well tolerated in both arms; grade ≥3 adverse events (AEs) were seen in 14 (54%) patients in Arm A and 6 (18%) patients in Arm B. The most common grade 3/4 AEs were gastrointestinal toxicities, neutropenia, thrombocytopenia and peripheral neuropathy. Serious AEs were seen in 11 (27%) patients in Arm A versus 6 (15%) patients in Arm B. Conclusions. Weekly and twice weekly bortezomib plus rituximab are active and well tolerated treatments for relapsed/refractory CD20+ indolent NHL. The more common weekly regimen appears to offer a similar response rate with less toxicity. The weekly regimen allowed delivery of a higher fraction of the intended dose, and a similar total cumulative dose, compared with the twice weekly regimen.

0188 CONSOLIDATION OF CHEMOTHERAPY RESPONSE IN MANTLE CELL LYMPHOMA PATIENTS WITH 90Y-BRUTUOMAB TIUXTETAN RADIOMUNOTHERAPY

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Background. MCL is an aggressive and prognostically unfavorable subtype of B-cell NHL, with a 5-y survival rate <20%. Standard chemotherapy for MCL includes fludarabine, cyclophosphamide, and mitoxantrone (FCM). Addition of rituximab (R) to FCM increases the overall response rate (ORR) from 46% to 58%. Conventional non-myeloablative RIT has been unsuccessful in MCL patients, with a large tumor burden and no initial cytoreduction. Aims: The Polish Lymphoma Research Group Trial (PLRG MCL1) assessed whether 90Y-Zevalin would consolidate the response achieved from FCM-R and provide better ORRs and longer TTP. Methods: 22 MCL pts (stage III-IV) not suitable for SCTs were grade ≥3 AEs were gastrointestinal toxicities, neutropenia, thrombocytopenia and peripheral neuropathy. Serious AEs were seen in 11 (27%) patients in Arm A versus 6 (15%) patients in Arm B. Conclusions. Weekly and twice weekly bortezomib plus rituximab are active and well tolerated treatments for relapsed/refractory CD20+ indolent NHL. The more common weekly regimen appears to offer a similar response rate with less toxicity. The weekly regimen allowed delivery of a higher fraction of the intended dose, and a similar total cumulative dose, compared with the twice weekly regimen.

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based on initial PLT count; max dose=32 mCi). ORR (CR+PR) was determined along with hematologic toxicity. Response is monitored at 6 wks, 3 mo, and 5-mo intervals for up to 2 yrs to assess TTP. Results: Following 90Y-Zevalin consolidation, 17 of 22 pts achieved a CR (no palpable lymph nodes or measurable masses on CT) and 13 of the 17 shifted from a PR after FCM/R to a CR. Three pts achieved a greater PR after 90Y-Zevalin consolidation. In 14 pts, CR plus the CR, 1 pt progressed at 3 mo, and another progressed immediately despite 90Y-Zevalin. Overall projected 1-year PFS is >85%. Most pts experience a 7-10-fold in stem and progenitor cells' clonogenic capacity, preceding a drop of WBCs and PLTs ≤50 wks after 90Y-Zevalin (cytopenias lasting 5-7 wks). In 3 pts, the times for FNM >1000/µl were ≤7-12 wks and for PLT levels ≤50,000/µl were ≤2-7 wks. In 3 pts, the time to transudation of the fluid to pts developed serious infections. Minimal, transitory (reversible by 2 wks) impairment of stromal cells was noted. Serum GM-CSF levels ≥2-fold at wk 4, and TPO and IL-3 ≥30% and 3-fold, respectively, at wk 2. EPO ≥3-fold during the first 4 wks and paralleled decreases in BFU-Es. Conclusions: 90Y-Zevalin consolidated the therapeutic benefit of FCM-R in 22 MCL pts. Fludarabine, a known radiosensitizer, reduces tumor burden before RIT and enhances the therapeutic benefit of non-myeloablative doses of 90Y-Zevalin, but may also induce the toxicity from RIT. Although 90Y-Zevalin has negative effects on stem and early progenitor cells, 90Y-Zevalin can be given safely after fludarabine-based therapy to high-risk MCL pts.

Background. MT103 is an anti-CD19/anti-CD3 bispecific single-chain antibody construct. Preclinical studies have shown that low picomolar antibody concentrations of MT103 can redirect unstimulated human T cells against CD19-positive human B lymphoma and normal B cells leading to their efficient lysis. MT103 is further characterized by mounting a T cell response, an activity at very low effector to target cell ratios, and an apparent independence of T cell costimulation. Correlation of polyclonal T cell response, an activity at very low effector to target cell ratios, and an apparent independence of T cell costimulation. Correlation of polyclonal T cell response, an activity at very low effector to target cell ratios, and an apparent independence of T cell costimulation. Correlation of polyclonal T cell response, an activity at very low effector to target cell ratios, and an apparent independence of T cell costimulation.

0189

MT103 (ANTI-CD19 X ANTI-CD3 BITE) INDUCES B CELL DEPLETION, CLEARANCE OF BONE MARROW INFILTRATION AND CLINICAL RESPONSES IN HIGH-RISK NHL PATIENTS: FIRST DATA FROM DOSE-ESCALATION STUDY MT103-104


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Background. MT103 is an anti-CD19/anti-CD3 bispecific single-chain antibody construct. Preclinical studies have shown that low picomolar concentrations of MT103 can redirect unstimulated human T cells against CD19-positive human B lymphoma and normal B cells leading to their efficient lysis. MT103 is further characterized by mounting a T cell response, an activity at very low effector to target cell ratios, and an apparent independence of T cell costimulation. Corticosteroids were co-administered as anti-inflammatory agents. Initial findings have shown that MT103 has an estimated half-life of approximately two hours. Here we describe a continuous infusion regimen. Aims. The MT103-104 study was set up to explore the safety and tolerability of increasing doses of MT103 given as continuous infusion. Additional objectives include the assessment of MT103 continuous infusion PK profile, the collection of pharmacodynamic data and the observation of clinical activity. Methods. Patients with relapsed indolent NHL were included according to a classical 3+3 dose escalation design with initial MT103 doses of 0.5 mg/m²/24h with initial steroid coverage. Safety and tolerability were assessed by CTC-AE criteria and dose escalation was only allowed after a data review committee (DRC) concluded safety of the previous dose with a DLT observation period of 14 days. Biological activity was monitored by investigating levels of systemic cytokines using specific ELISAs and by quantification and characterisation of peripheral immune cell subsets via FACS analysis. After 4 weeks of MT103 treatment, a control CT scan was performed. If patients were at least stable according to standardized Cheson criteria (reviewed by central radiology), an additional 4-week cycle of MT103 was offered to the patients. Results. As of today, 19 patients with a median number of 4 previous chemo-/immuno-therapies have been included into MT103-104. During dose-escalation from DL1 (0.5 mg/m²/24h) up to DL3 (5 mg/m²/24h) no dose-limiting toxicity was observed, and AEs were generally moderate. At DL4 (5 mg/m²/24h on the first day, 15 mg/m²/24h as maintenance dose), 7 patients were treated with 2 patients receiving less than 14 days of treatment. One patient experienced elevation of liver enzymes up to CTC grade 3 after 2 weeks, which recurred upon re-administration of MT103, and 1 patient experienced confusion and disorientation on the second day of treatment. Depletion of circulating B (lymphoma) cells by end of cycle was obtained in 6/7 evaluable patients (with treatment for >2 weeks and B cells detectable in peripheral blood prior to MT108 infusion) with a dose-dependent increase in frequency that reached 100% depletion at DL4. At DL4, 3 of 7 patients had significant bone marrow (BM) infiltration (>10%) with 1 patient showing reduction of and 2 patients showing complete disappearance of lymphoma cells in BM. Best overall tumour response in the 14 evaluable patients (with treatment for >2 weeks and scanning of all involved areas) was 1 CR (at DL4), 2 PR (at DL4), 1 MR, 7 SD and 5 PD. Summary. These preliminary results observed in indolent NHL patients clearly indicate single agent biological and clinical activity of MT108. Further evaluation of dose and schedule is ongoing.

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PKC-BETA II EXPRESSION HAS PROGNOSTIC IMPACT IN NODAL DIFFUSE LARGE B-CELL LYMPHOMA

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Background. Recent studies of gene expression in diffuse large B-cell lymphomas (DLBCL) have identified gene signatures associated with germinal center or post-germinal center lymphocytes. These signatures have been shown to have prognostic significance in DLBCL, and to correlate with similar classifications based on immunohistochemical biomarkers. Protein kinase C β II (PKC-β II) was characterized as one such gene, and recent studies have suggested that its expression is associated with a poor prognosis. Aim. To determine the prognostic significance of the expression of PKC-β II in patients with nodal DLBCL. Methods. Patients with de novo nodal DLBCL treated in two hospitals were enrolled retrospectively. Inclusion criteria for treatment were treatment with conventional chemotherapy regimens, HIV-negative status, and tissue availability. Diagnosis was confirmed by a pathologic review board. Clinical data were obtained from the charts. The IPI was grouped in low-risk (0-2 factors) and high-risk (3-5 factors). Formalin-fixed, paraffin-embedded tissues were stained with a monoclonal antibody against PKC-β II protein (Sigma, P-5205). Cases with more than 10% stained large cells were considered positive. Results. A total of 125 patients were enrolled. The median follow-up was 5.3 years for the surviving patients. Data were available for IPI classification in 118 patients, and 83 patients were in the low-risk group. Forty-eight patients (38%) were positive for PKC-β II. There were no differences in LDH, age, B symptoms, Ann Arbor stage or IPI group according to the expression of PKC-β II. More females than males were PKC-β II positive (54% vs 27%, p=0.003). Complete remission was obtained in 70%, and was not influenced by PKC-β II status (67% vs 71%). The 5-year event-free survival (EFS) was shorter in high-risk patients (14% vs 59%, p<0.001) and in those with PKC-β II positivity (56% vs. 49%, p=0.054). Only the IPI influenced the 5-year overall survival (18% vs. 70%, p<0.001). However, in low-risk patients, PKC-β II expression was related to a shorter 5-year OS (60% vs. 76%, p=0.03) and a shorter 5-year EFS (48% vs. 66%, p=0.014). In a Cox regression analysis for EFS, PKC-β II expression (hazard ratio = 1.6, p=0.04) and the IPI (HR=3.06, p=0.001) were independent predictors for poor survival. PKC-β II (HR=1.72, p=0.046) and the IPI (HR=5.16, p<0.001) were also independent prognostic factors for the OS. Conclusion. PKC-β II expression, along with the IPI, was associated with a shorter EFS and OS in patients with nodal DLBCL. PKC-β II identified a subgroup of patients, within the IPI low-risk group, who had a shorter OS.

0191

CIGARETTE SMOKING AND ALCOHOL CONSUMPTION AS DETERMINANTS OF SURVIVAL IN NON-HODGKINS LYMPHOMA: A POPULATION-BASED STUDY

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Background. The risk of non-Hodgkin’s Lymphoma (NHL) seems to be enhanced by cigarette smoking and lowered by alcohol drinking. No study has ever focused on the role of these factors on survival from NHL. Aims. To assess whether cigarette smoking and alcohol drinking affect NHL survival Methods. A population-based prospective study on 1,185 NHL patients, diagnosed between 1991 and 1993, followed-up until 2002, was carried out. At diagnosis, clinical and socio-demographic data were recorded and lifestyle habits were assessed through a validated questionnaire. Survival analysis was performed with Kaplan-Meier
Methods. Hazard ratios (HR) were estimated by Cox regression. Results. The mean follow-up was 6.6 years (std.dev 4.3). The mean survival time was 7.56 years (std.dev 0.155). At both univariate and multivariate analysis heavy cigarette smoking and alcohol drinking were associated with poor survival. Compared with those with a lower cumulative exposure to tobacco smoking, those who had smoked ≥31 pack-years had a worse survival (HR=1.60, 95%CI=1.18-2.16). Drinkers had a higher risk of death than non-drinkers (HR=1.10-1.81). Considering only those who had NHL as cause of death, the HR for the higher category of pack-years smoked, compared with the lowest, was 1.65 (95%CI=1.18-2.33) and for drinkers, compared with non-drinkers, it was 1.83 (95%CI=1.01-1.80). Conclusions. cigarette smoking and alcohol drinking may influence NHL survival.

0192
IBRITUMOMAB TIUXETAN COMBINED WITH HIGH-DOSE CHEMOTHERAPY AND AUTOLOGOUS STEM-CELL TRANSPLANTATION IN PATIENTS WITH CHEMO-REFRACTORY AGGRESSIVE NON-HODGKIN'S LYMPHOMA

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Background. High-dose chemotherapy and autologous stem-cell transplantation (SCT) have an established role in the treatment of patients with first chemo-sensitive relapse of aggressive lymphoma. However, autologous SCT has only limited success when performed in refractory or progressive stage of the disease and the expected 1-year progression-free survival (PFS) in this setting is less than 20%. The major cause of treatment failure is disease relapse.

Aims. This study was designed to explore the safety and outcome following inclusion of Zevalin in the treatment regimen.

Methods. Patients were eligible for this study only if they had refractory lymphoma and a positive PET-CT prior to SCT. Rituximab 250 mg/m² followed by Zevalin 0.4 mCi/kg were given on day -14. Chemotherapy according to standard BEAM regimen was started on day -6. Results. The study included 22 patients, 14 men and 8 women, median age 55 years (range, 35-66). Histology was diffuse large cell (n=15), transformed follicular (n=6) and mantle cell lymphoma (n=1). Patients had active lymphoma at SCT, either primary refractory (n=12) or refractory relapse (n=10) and 12 patients had bulky disease at SCT. The median number of prior lines of therapy was 3 (range, 1-6). There were no early infusion reactions associated with Zevalin. Fifteen patients achieved CR (3 of them after additional radiotherapy given after SCT), 5 achieved PR and 2 died early after SCT from organ toxicities. With a median follow-up of 9 months (range, 1-21), 15 patients are alive and 7 have died. The estimated 1-year survival is 54% (28-81). Only 4 patients relapsed so far with a 1-year cumulative incidence of only 22% (9-53). As expected in this group of patients with refractory disease all relapses occurred within 4 months of SCT, such that despite the relative short follow-up relapse rate seems lower than expected. Two pts died of multi-organ toxicities and 2 of late occurring infections. The day 100 treatment-related mortality was 9%. These rates of non-relapse mortality are lower than other reported series. The inclusion of Zevalin in the conditioning regimen given prior to autologous SCT may reduce the risk of post SCT relapse and improve the outcome of patients with refractory lymphoma given SCT with standard regimens. This observation merits further study in larger comparative studies.

0193
CENTRAL NERVOUS SYSTEM INVOLVEMENT IN PATIENTS WITH NON-HODGKIN’S LYMPHOMA. INCIDENCE AND CLINICAL CHARACTERISTICS


Institut Catat d’Oncologia, L’HOSPITALET DE LLLOBREGAT, Cancer Institute

Purpose. The aim of the study was to evaluate the incidence of central nervous system infiltration in patients with Non-Hodgkin’s lymphoma diagnosed according to the REAL/WHO classification and to describe the clinical features and treatment outcome. Patients and Methods. All patients diagnosed of a lymphoid neoplasm in our centre between May 1994 and May 2004 (n=2544) were included in our analysis. We identified all the cases with CNS infiltration at diagnosis or during the clinical course and excluded those who received intrathecal prophylaxis. We evaluated the incidence, clinical characteristics and response to treatment. Results. Forty (3.8%) patients with CNS infiltration were identified. Twenty one (52.5%) males, with a median age of 56 years (range 31-82 years). Ten (25%) patients presented CNS infiltration at diagnosis. Thirty patients (75%) developed CNS involvement during the course of the disease at a median time of 12 months (range 3-4.20.5m) from initial diagnosis. Four (10%) were HIV+ patients. CNS infiltration due to REAL/WHO subtypes is shown in the table below. Clinical and biological findings at diagnosis were as follows: IVL (n=55%), extranodal involvement: 36 (90%), bone marrow infiltration: 15 (40.5%), bulky disease: 20 (50%), B symptoms: 14 (35%) and ECOG ≥ 2: 20 (50%). Response to treatment: CR 10 patients (27%), PR 5 (13.5%) and failure 22 (59.5%). Median overall survival was 2.26 months (range 0.72-3.8 months). Conclusions. The high incidence of CNS involvement in lymphoma patients although previous CNS prophylaxis, makes us hypothesise that unknown factors could be associated to this phenomenon. Treatment response and survival of these patients is poor.

<table>
<thead>
<tr>
<th>Entity</th>
<th>CNS Infiltration/Lymphoid Neoplasm</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diffuse large B cell lymphoma</td>
<td>26/368</td>
<td>7.1%</td>
</tr>
<tr>
<td>Follicular lymphoma</td>
<td>6/171</td>
<td>3.5%</td>
</tr>
<tr>
<td>Multiple myeloma</td>
<td>3/238</td>
<td>1.3%</td>
</tr>
<tr>
<td>Splenic marginal zone lymphoma</td>
<td>2/108</td>
<td>2.0%</td>
</tr>
<tr>
<td>Mantle cell lymphoma</td>
<td>1/60</td>
<td>1.7%</td>
</tr>
<tr>
<td>Cutaneous T cell lymphoma</td>
<td>1/88</td>
<td>1.1%</td>
</tr>
<tr>
<td>Anaplastic lymphoma</td>
<td>1/10</td>
<td>1.0%</td>
</tr>
<tr>
<td>Total</td>
<td>40/1043</td>
<td>3.8%</td>
</tr>
</tbody>
</table>

0194
CLINICAL FEATURES OF THE WESTERN AND ASIAN FORMS OF INTRAVASCULAR LYMPHOMA (IVL) VARY ACCORDING TO THE PRESENCE OF HEMOPHAGOCYTIC SYNDROME (HPS) AND NOT TO THE GEOGRAPHICAL AREA


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Published data suggest the existence of some clinical differences between IVL patients diagnosed in Asian and Western countries. Aim. To explore potentially different clinical forms of IVL by comparing the clinical features of the largest cumulative series of IVL patients diagnosed in Western countries and three subgroups of IVL patients diagnosed in different Asian countries and published in the English literature. Methods. Clinical records and pathological material of 45 HIV-negative patients with IVL diagnosed in 8 Western Countries (Western-IVL) were reviewed. Clinical features of this series were compared with 282 previously reported cases of IVL diagnosed in Western countries (Western-IVL) and with 120 previously reported cases of IVL diagnosed in Japan (n=66) and other Asian Countries (n=54). Analysis was performed according to the presence of HPS. Results. HPS was absent in our patients; it was diagnosed in 5 (2%) previously reported cases of Western-IVL (p=0.57), in 36 (44%) Japanese patients (p=0.00001) and in 4 cases (12%) diagnosed in other Asian countries (p=0.03). Analysis of differences in clinical presentation and laboratory findings included four subgroups of IVL: 45 Western-IVL patients, 38 Japanese patients with IVL and HPS (J-HPS), 48 Japanese patients with IVL without HPS (J-IVL) and 30 patients with IVL with HPS diagnosed in Asian countries other than Japan (Eastern-IVL). Median age was very similar among studied subgroups, oscillating between 62 and 69 years, with a constant slight prevalence among males. As reported in the table, there were no significant differences in presenting symptoms, sites of disease or laboratory findings among Western-IVL, J-IVL and Eastern-IVL patients. Conversely, stage-IV disease, fever of unknown origin, involvement of liver, spleen, bone marrow or lung, fatigue, jaundice, thrombocytopenia, increased serum levels of hepatic enzymes as well as a concomitant non-relapse mortality are more common among the
J-HPS patients in comparison with the other groups. Conversely, skin and central nervous system involvement was significantly more rare in J-HPS patients. No significant differences were observed in terms of anemia, leucopenia, monoclonal component, and peripheral blood involvement. In patients treated with anthracycline-based chemotherapy (21 from our Western-IVL series and 27 from J-HPS series), complete remission rate was 52% and 58% (p = 0.93), with a 2-year overall survival of 45±11% and 22±5% (p = 0.04), respectively. Conclusions. The association between IVL and HPS is anecdotal diagnosed outside of Japan. IVL significantly varies in clinical features and laboratory findings according to the presence of HPS and to the geographical area. Patients with IVL but without HPS diagnosed in Western countries, Japan and other Asian countries display similar characteristics and could be considered as forming part of a classical form of IVL. J-HPS patients display numerous clinical differences with respect to the classical form and could be considered as a HPS-related variant of IVL. When treated with anthracycline-based chemotherapy, both variants exhibit a worse prognosis, specially in HPS-related cases; thus, rendering advisable treatment intensification. An extensive phenotypic and molecular characterization is needed to confirm whether these clinical differences might reflect discordant biological entities within IVL.

### Table 0195

<table>
<thead>
<tr>
<th>Western (n=45)</th>
<th>J-HPS (n=38)</th>
<th>p</th>
<th>J-IVL (n=48)</th>
<th>Eastern (n=50)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>19 (42%)</td>
<td>34 (89%)</td>
<td>0.0001</td>
<td>20 (42%)</td>
<td>0.96</td>
</tr>
<tr>
<td>Stage IV</td>
<td>34 (76%)</td>
<td>37 (97%)</td>
<td>0.004</td>
<td>45 (94%)</td>
<td>0.19</td>
</tr>
<tr>
<td>Skin</td>
<td>17 (38%)</td>
<td>3 (3%)</td>
<td>0.0011</td>
<td>12 (25%)</td>
<td>0.18</td>
</tr>
<tr>
<td>CNS</td>
<td>18 (40%)</td>
<td>8 (21%)</td>
<td>0.034</td>
<td>24 (51%)</td>
<td>0.24</td>
</tr>
<tr>
<td>Liver</td>
<td>11 (22%)</td>
<td>25 (66%)</td>
<td>0.0015</td>
<td>31 (63%)</td>
<td>0.63</td>
</tr>
<tr>
<td>Spleen</td>
<td>11 (22%)</td>
<td>11 (24%)</td>
<td>0.0011</td>
<td>10 (33%)</td>
<td>0.68</td>
</tr>
<tr>
<td>Lymph</td>
<td>4 (9%)</td>
<td>2 (9%)</td>
<td>0.68</td>
<td>4 (9%)</td>
<td>0.40</td>
</tr>
<tr>
<td>Lung</td>
<td>8 (18%)</td>
<td>14 (31%)</td>
<td>0.037</td>
<td>13 (27%)</td>
<td>0.28</td>
</tr>
<tr>
<td>B. marrow</td>
<td>14 (31%)</td>
<td>28 (74%)</td>
<td>0.016</td>
<td>16 (33%)</td>
<td>0.82</td>
</tr>
<tr>
<td>Thrombocytosis</td>
<td>16 (36%)</td>
<td>26 (66%)</td>
<td>0.005</td>
<td>6 (34%)</td>
<td>0.07</td>
</tr>
<tr>
<td>High LDH</td>
<td>29/54 (47%)</td>
<td>36 (69%)</td>
<td>0.17</td>
<td>31 (61%)</td>
<td>0.43</td>
</tr>
<tr>
<td>High ALT</td>
<td>10 (20%)</td>
<td>11 (22%)</td>
<td>0.03</td>
<td>3 (6%)</td>
<td>0.71</td>
</tr>
<tr>
<td>High bilirubin</td>
<td>4 (9%)</td>
<td>11 (22%)</td>
<td>0.04</td>
<td>0 (0%)</td>
<td>0.23</td>
</tr>
</tbody>
</table>

From October 2004 to January 2006, 75 newly diagnosed patients with grade 1-2 follicular lymphoma (FL) were included in a prospective study designed to evaluate clinical efficacy, evaluated as time to progression, after 6 cycles of a combined treatment with rituximab (25 mg/m² on 3 days), cyclophosphamide (1 g/m²/day1), and rituximab (375 mg/m²/week). 10% cycles (31% pts) were dose reduced (375 mg/m²/day1) and rituximab (375 mg/m²/day1) (FCR) followed by maintenance treatment with rituximab (375 mg/m²/week) X4 weeks/6 months/2 years). Clinical and molecular response rates as well as safety of this combination were also evaluated. We present results from the first 32 patients that completed at least 4 induction cycles. Median age was 57 years (30-74), 91% of the patients were in stages I-IV, 25% had bulky disease (>7 cm), and extranodal disease was present in 85%. After 4 cycles all patients responded and 73% obtained a CR or uCR. Bcl2/IgH MBR and MCR rearrangements were studied by real time PCR and VDJ IgH, IgL Kappa VJ, K deleting population at diagnosis was identified in 62,5% of patients. 25 patients with a monoclonal population at diagnosis were studied after induction treatment and all patients obtained a complete molecular response. 15% of patients presented at least one serious adverse event being neutropenia the most common. Toxicity from the FCR regimen was observed specially among patients older than 60 years. Two cases of opportunistic infections were observed (CMV disease and cerebral toxoplasmosis). A profound and prolonged lymphopenia occurred in the majority of patients as well as some cases that developed a delayed neutropenia persisting during months after complete the induction treatment. We have observed that FCR regimen has a potent antitumoral activity in newly diagnosed patients with FL and all patients evaluable at molecular level have obtained a complete response; nevertheless profound immunosuppression developed in some cases resulting in the development of opportunistic infections. Data from the 75 included patients will be actualized and presented at the time of the meeting.
**0197**

**INTENSIVE CHEMOTHERAPY (HIGH-DOSE CHOP/ESHAP REGIMEN) FOLLOWED BY AUTOLOGOUS STEM-CELL TRANSPLANTATION IN PREVIOUSLY UNTREATED PATIENTS WITH PERIPHERAL T-CELL LYMPHOMA**


1H. Clinic, BARCELONA, Spain; 2H. Clinic, BARCELONA, Spain; 3H. Germans Trias y Pujol, BARCELONA, Spain; 4H. Joan XXIII, TARRACONA, Spain; 5H. del Mar, BARCELONA, Spain; 6H. Mútua de Terrassa, TERRASSA (BARCELONA), Spain; 7H. Sant Pau, BARCELONA, Spain

**Background.** The outcome of patients (pts) with PTCL receiving conventional therapy is dismal. Because of this, there is an increasing interest to investigate intensive treatments in these pts. **Aims.** To analyze the results in terms of toxicity, response and outcome, of a phase II trial that includes high-dose chemotherapy (CT) plus ASCT as first line treatment for pts with PTCL. **Methods.** Forty pts (29M/11F; median age: 47 yrs) diagnosed with PTCL (excluding cutaneous and anaplastic ALK+), in stages II-IV and <65 yrs, who have finished the planned therapy, are the subject of this analysis. Pts received intensive CT (3 courses of high-dose CHOP [cyclophosphamide 2000 mg/m2 day 1, adriamycin 90 mg/m2 day 1, vincristine 2 mg day 1, prednisone 60 mg/m2/day, days 1 to 5, mesnum 150% of cyclophosphamide dose, G-CSF 300 µg/day days 7 to 14], alternating with 3 courses of standard ESHAP). Responders (CR or PR) were submitted to ASCT. **Results.** Twenty-three patients had a PTCL unspecified, nine angioimmunoblastic, two panniculitic and six other subtypes. Eleven pts (28%) presented with primary extranodal disease, 20% in stage IV, and 14% (35%) had bone marrow involvement. Forty five percent of the pts had high/intermediate or high-risk IPI, whereas 49% were in the groups 3 or 4 according to the Italian Index for PTCL. Twenty seven pts (68%) received the planned 6 courses of CT. Response rate after CT was: CR, 19 cases (47.5%); PR, 4 (10%); failure, 17 (42.5%), including one pt who died because of sepsis. Hematologic toxicity of CT mainly consisted of neutropenia (grades 3-4 in 97 and 62% after high-dose CHOP and ESHAP, respectively) and thrombocytopenia (grades 3-4 in 63 and 68%, respectively). Severe infection requiring hospitalization was observed in 38 and 15% of courses of high-dose CHOP and ESHAP, respectively. Only 16 of the 23 candidates (70% of all candidates and 40% of all pts) received ASCT due to the lack of stem-cell mobilization (3 cases), severe previous toxicity (2), early relapse (1) and pt decision (1). No differences in the outcome were seen among these 23 pts according to whether or not they eventually received ASCT. No major toxicity was observed after ASCT. Response after the whole treatment was: CR, 20 cases (50%); PR, 5 (8%); failure, 17 (42%). Two of 14 pts in CR and 2 pts in PR eventually progressed. Four-year failure-free survival (FFS) was 58% (95% CI: 14-46%), whereas 4 yr event-free survival for pts achieving CR was 63% (95% CI: 45-89%). Twenty-one pts have died during follow-up, with a 4-yr overall survival (OS) of 40% (95% CI: 21-55%). Most patients died because of PTCL progression, but 2 died in CR due to secondary leukemia and lung cancer, respectively. Both the IPI and the Italian Index were able to predict FFS and OS. **Conclusion.** In this series of patients with PTCL, a relatively high CR rate was obtained with high-dose CHOP/ESHAP followed by ASCT. Toxicity was manageable. However, the prognosis of patients with PTCL, particularly of those not achieving CR, is still very unfavorable.

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**0198**

**THE ADDITION OF RITUXIMAB TO CHEMOTHERAPY MAY CHANGE PROGNOSTIC FACTORS, INCLUDING TUMOR BCL-2 EXPRESSION, IN PATIENTS WITH DIFFUSE LARGE B CELL LYMPHOMA**


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**Background and Aim.** Prognostic factors may change as a result of the introduction of better therapies. In this regard, the addition of rituximab to standard chemotherapy has highly improved the outcome of patients (pts) with diffuse large B-cell lymphoma [DLBCL]. The aim of the present study was to assess the outcome and prognostic factors, including bcl-2 tumor expression, in pts with DLBCL before and after the rituximab era. **Patients and Methods.** 131 pts (median age: 58 yrs; 77M/54F) diagnosed with DLBCL in a single institution between January 1997 and January 2005 were treated with CHOP-like regimens [CHOP] until Jan 2002 (N=60; median follow-up: 5.8 yrs) and with CHOP-like plus rituximab [R-CHOP] since that date (N=71; median follow-up: 1.7 yrs). The only criterion to include pts was the availability of histological material. Tumor bcl-2 expression was considered positive when superior to 25%. Median overall survival was 3.4 years. Main clinicobiological features were assessed for prognostic value. **Results.** Main initial characteristics, including IPI and bcl-2 expression, were similar (p=0.1) between patients receiving or not rituximab. CR rate was 50% and 71% for pts treated with CHOP and R-CHOP, respectively (p=0.01). 3-yr overall survival [OS] was of 56% (95% CI: 46-66) and 92% (95% CI: 83-100) for pts CHOP and R-CHOP, respectively (p=0.002). Age, performance status, LDH and IPI predicted OS both in the whole series and in the two treatment subgroups. Pts receiving CHOP with bcl-2 positive expression showed poorer OS than those bcl-2 negative (3-yr OS: 28 vs. 67%, respectively; p=0.02). On the contrary, no difference in terms of OS was observed according to bcl-2 expression in the group of pts treated with R-CHOP (figure). In the multivariate analysis, IPI (p=0.001), treatment (CHOP vs. R-CHOP) (p=0.01) and bcl-2 expression (p=0.02) were the most important variables predicting OS. These figures were similar in the group of pts treated with CHOP, whereas only IPI maintained prognostic interest in the subset of pts treated with R-CHOP. **Conclusion.** The addition of Rituximab to chemotherapy improves the outcome and changes prognostic factors, including the negative impact of Bcl-2 expression, in pts with DLBCL. More studies are warranted to assess changes in other biological predictive indicators.
Non-Hodgkin’s Lymphoma - Clinical II

**0199**

**CLINICAL OUTCOME OF LOW GRADE NON Hodgkin’s Lymphoma PATIENTS WITH BONE MARROW INVOLVEMENT DETECTED BY FLOW CYTOMETRY ALONE**

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**Background.** BM involvement in low grade NHL patients results in stage IV clinical classification and has a negative impact on survival. The standard practice is morphologic examination of BM biopsy conducted at diagnosis. In many institutions flow cytometry (FC) is also routinely performed on BM aspirate samples accompanying respective biopsies. FC is believed to increase the sensitivity of the morphologic analysis by detecting occult lymphoma cells evading the pathologist’s eyes. However, the prevalence of such finding and especially its clinical significance are largely unknown. In our institute bone marrow biopsies of NHL patients conducted after 1993 were routinely accompanied by bone marrow aspirates with FC analysis. Aims. We aimed to analyze the prevalence and clinical significance of BM FACS findings in patients with low grade NHL. Methods. We retrospectively reviewed the charts of all low grade NHL patients (small lymphocytic lymphoma, follicular small cleaved cell NHL, follicular mixed small and large cell, marginal zone B-cell lymphoma, mantle cell lymphoma and Waldenstrom macroglobulinemia) diagnosed or followed in the Hematology Unit between 1994 and 2004, who had undergone bone marrow biopsies and aspirates as a part of their diagnostic workup or before treatment. Flow cytometric results were considered positive if they showed either a ratio of immunoglobulin light chain expression of kappalamda >3:1 or lambdakappa >2:1 in at least 2% of the gated population. Selected cells were analyzed by two or three color combinations: CD5 versus CD19, CD20 versus CD10, and kappa light chain versus lambda light chain occasionally with the addition of CD19 or CD20. Results. Lymphoma involving BM by morphology was found in the biopsies of 48 patients (61.4%) (BM+ group). Of the remaining 1 patient had inconclusive results and 26 patients had normal BM biopsies. Of these 27 patients the FC analysis was positive in 9 patients (BM-FC+ group) and negative in 18 (BM-FC- group). We could not compare the groups using FLIPI or IPI scores as a whole since both include the stage as one of the five summed parameters while BM involvement was different by definition between the groups. However, the groups had similar parameters that are prognostically important and are part of the FLIPI scoring system including age, hemoglobin and LDH levels and also the number of involved extranodal sites. Splenic involvement and number of involved nodal sites were higher in BM+ and BM-FC+ groups than in BM-FC- group. Significant differences in disease progression as indicated by time-to-treatment were observed. The median treatment-free period was shorter in the BM+ and BM-FC+ groups (1 month and 4 months, respectively) as compared with the BM-FC- group (31 months) (log rank test =0.0195). BM-FC-patients had significantly longer survival time than BM+ and BM-FC+ groups. Median survival time was not reached for the BM-FC- patients while in the BM+ and BM-FC+ groups median survival times were 129 and 89 months respectively with no significant difference between them. Conclusions. We conclude that the outcome of low grade NHL patients found to have malignant cells by FC analysis while their BM morphology is normal is the same as that of patients with histological involvement. This may imply that patients with localized disease who have bone marrow involvement by FC should be regarded as advanced stage disease.

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**0200**

**CHOP (Doxorubicin) CHEMOTHERAPY IS SUPERIOR TO CNOP (Mitoxantrone) IN THE TREATMENT OF PATIENTS WITH AGGRESSIVE NON-HODGKIN Lymphoma (Review)**

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**Introduction.** Mitoxantrone (M), an anthracenedione, was introduced in the early/mid 1980s as a more tolerable alternative to anthracyclines. This agent has a broad anti-tumour activity including lymphoma with potentially less cardiotoxicity than doxorubicin (D), which may be of particular importance in the elderly patient population. However, an important issue is whether M is as efficacious as D in the treatment of NHL patients. Methods. Through search of several relevant databases and direct contacts with lymphoma investigators worldwide, we identified seven randomised studies of previously untreated patients comparing CHOP and CNOP chemotherapy in aggressive NHL. In this analysis we included five trials where (D; 50 mg/m2) was compared with (M;10-12 mg/m2); table) and the interval between chemotherapy courses was 3-4 weeks. Patients reported in the Pavlovsky article were included in the Bezwooda report, why analyses were performed with and without patients reported by Bezwooda et al. Odds ratios of complete remission (CR) and overall survival (OS) were pooled using a fixed effects model. Results. CHOP was significantly superior to CNOP with regard to both CR rate and OS (Figures 1-2).

**Table. Randomised trials comparing doxorubicin with mitoxantrone in untreated patients with aggressive NHL.**

<table>
<thead>
<tr>
<th>Reference Regimen</th>
<th>Doses of doxorubicin/ mitoxantrone (mg/m²) (weeks) (years)</th>
<th>Treatment Number of patients</th>
<th>Median Number of CR rate Overall survival</th>
<th>Reference Regimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osby CHOP 50</td>
<td>3</td>
<td>205</td>
<td>71</td>
<td>205</td>
</tr>
<tr>
<td>et al., 2003 CHOP</td>
<td>10</td>
<td>203</td>
<td>70</td>
<td>303</td>
</tr>
<tr>
<td>Bezwooda CHOP 50</td>
<td>3</td>
<td>164</td>
<td>55</td>
<td>69</td>
</tr>
<tr>
<td>et al., 1995 CHOP</td>
<td>10</td>
<td>103</td>
<td>54</td>
<td>70</td>
</tr>
<tr>
<td>Sanerwold CHOP 50</td>
<td>4</td>
<td>72</td>
<td>70</td>
<td>72</td>
</tr>
<tr>
<td>et al., 1995 CHOP</td>
<td>10</td>
<td>76</td>
<td>71</td>
<td>76</td>
</tr>
<tr>
<td>Pavlovsky CHOP 50</td>
<td>3</td>
<td>44</td>
<td>NI</td>
<td>NI</td>
</tr>
<tr>
<td>et al., 1992 CHOP</td>
<td>10</td>
<td>45</td>
<td>NI</td>
<td>NI</td>
</tr>
<tr>
<td>Groganov CHOP 50</td>
<td>3-4</td>
<td>20</td>
<td>57°</td>
<td>NI</td>
</tr>
<tr>
<td>et al., 1988 CHOP</td>
<td>12</td>
<td>15</td>
<td>47</td>
<td>NI</td>
</tr>
</tbody>
</table>

*p<0.05; **p<0.01; ***p<0.001; n.s. = Not significant; NI = No information; ° Approximately (visual reading); Mean; p<0.05

Myelosuppression was not more severe using CHOP, rather the opposite. However, the incidence of gastrointestinal toxicity and alopecia was significantly lower in patients treated with CNOP. Conclusion. CHOP chemotherapy is more efficacious than CNOP at equitoxic (myelosuppression) doses leading to higher CR rates and improved survival.
0201
EARLY-MID TREATMENT C-REACTIVE PROTEIN LEVELS PREDICT TIME TO DISEASE PROGRESSION OR RELAPSE AS WELL AS OVERALL SURVIVAL IN AGGRESSIVE NON-HODGKIN’S LYMPHOMA

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Background. Higher pretreatment serum CRP levels in patients with aggressive non-Hodgkin’s lymphoma (NHL) are associated with a more aggressive histology, B-symptoms and a shorter overall survival (OS). In most patients who achieve complete remission (CR) at the end of therapy, serum CRP levels appear to return to normal range. In the light of an emerging role for early-mid treatment FDG-PET as an important prognostic indicator for progression free survival (PFS) and OS in NHL, we considered whether a simple parameter, such as early-mid treatment CRP, could also be a significant prognostic factor in this respect. Aims. To evaluate the possibility that wide ranged CRP could predict early response to treatment, time to progression or relapse and overall survival in aggressive NHL.

Patients and Methods. Serum CRP levels were monitored in fifty five patients with aggressive NHL (newly diagnosed and relapsed) at baseline and before receiving each of the next 5 chemotherapy cycles. The lowest value of the early mid-term CRP levels recorded was compared to the interim FDG-PET results, as well as to the clinical course and outcome. Results. At baseline, patients with aggressive NHL presenting with B-symptoms or bulky disease had higher pretreatment CRP levels compared to those recorded in asymptomatic patients and those with non-bulky disease (mean 90±71 mg/L vs 37±149, p=0.0013 and mean 76.8±54.2 vs 40.3±55.9 mg/L, p=0.04, respectively). Pretreatment CRP levels ≥20 mg/L were also associated with a shorter overall survival (p=0.029). During chemotherapy, the lowest value of early-mid treatment CRP levels significantly predicted the results of the interim FDG-PET (p=0.04 with a hazard ratio of 1.28). This implies that any increase of 1 mg/L in the serum CRP level enhances the risk of a positive FDG-PET scan by 12.8%. Moreover, patients who did not achieve an early-mid treatment CRP level of <5 mg/L, appear to have a shorter time to disease progression or relapse (p=0.001) and a reduced overall survival (p=0.016) (Figure 1). In multivariate analysis, both early-mid treatment CRP levels and interim FDG-PET findings significantly predicted PFS (p=0.02 and p=0.004, respectively), while OS was significantly predicted by the early-mid treatment CRP levels (p=0.016) and by the International Prognostic Index (IPI) (p=0.05). Conclusions. The early-mid treatment serum CRP level is an important prognostic factor in aggressive NHL. Patients who do not achieve an early-mid treatment level of <5 mg/L have faster disease progression or earlier relapse and also appear to have an inferior overall survival.

Figure 1. Kaplan-Meier curves of PFS and OS.

0202
BEAC OR BEAM CHEMOTHERAPY FOLLOWED BY AUTOLOGOUS STEM CELL TRANSPLANTATION IN NON-HODGKIN’S LYMPHOMA PATIENTS: COMPARATIVE ANALYSIS ON EFFICACY AND TOXICITY

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Background. Non-Hodgkin’s lymphoma (NHL) is the major indication of high dose chemotherapy (HDC) followed by autologous stem cell transplantation (ASCT). However, little is known on the comparative efficacy and toxicity of various HDC regimens. Aims. This study aimed to compare the efficacy and toxicity of BEAC and BEAM regimen. Methods. Between April 1994 and February 2005, 97 NHL patients were received HDC with BEAC (N=69) or BEAM (N=28) followed by ASCT at Asan Medical Center. We matched one patient received BEAM with two patients received BEAC who has same International Prognostic Index (IPI). Thus total 84 patients (56 in BEAC group and 28 in BEAM group) were analyzed. Results. Of 84 patients, 55 (65.5%) were male, 29 (34.5%) were female and median age was 40.5 (15-65) years. Baseline characteristics such as age, sex, disease status at ASCT, Histology, stage at ASCT, IPI were not different between two groups. Time to neutrophil engraftment (WBC >0.5×10^9/mm³) was significantly longer in BEAC group (p=0.0201) than in BEAM group (1.6±0.8 days vs. 1.0±0.3 days, p=0.037). Platelet engraftment (platelet >20×10^9/mm³) was faster and total amount of platelet transfusion was less in BEAM group. Patients received BEAM had more frequent WHO grade ≥2 diarrhea than those received BEAC (46.4% vs. 19.6%, p=0.010). Other clinically important toxicities such as mucositis, nausea/vomiting, bleeding were not different between two groups. In addition, neutropenic fever and documented infection were not different between two groups. Two year overall survival (OS) rate was 30% in BEAC group and 66% in BEAM group. Two year event free survival (EFS) rate was 34% in BEAC group and 61% in BEAM group. Both OS and EFS was significantly superior in BEAM group than in BEAC group (p=0.049, p=0.032, respectively). Summary/Conclusions. BEAM appears to be a superior HDC regimen in the aspect of OS and EFS than BEAC while regimen related toxicity is similar except more frequent diarrhea in BEAM.

0203
RAPID INFUSION OF RITUXIMAB WITH OR WITHOUT STEROID CONTAINING CHEMOTHERAPY. ONE YEAR EXPERIENCE IN A SINGLE CENTRE

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Background. Infusion-related toxicity is frequent after the administration of Rituximab despite the fact that strict guidelines have been recommended. Recently, a rapid rituximab infusion schedule in combination with a steroid containing chemotherapy regimen was well tolerated and safe. Aim. To assess the feasibility of a fast infusion of rituximab with or without steroid containing chemotherapy. Methods. Inclusion criteria: disease susceptible of treatment with rituximab and having been treated with a first infusion of rituximab according to the product monograph; Further infusions over a total of 314 infusions. Patient characteristics: median age 64 yr (range 28-87), 47% males, 39% DLBCL, 36% follicular 40%, mantle 6%, MALT 11%, other 7%. Number of rituximab infusions: 199 as treatment (combined or not with chemotherapy) and 115 as maintenance. Number of rituximab administrations with and without steroids: 123 and 191 infusions, respectively. Median time from previous rituximab infusion was 28 days (range 7-272). Sixteen rapid infusions were administered with an interval greater than 90 days from the previous standard infusions. This rapid rituximab administration schedule was very well tolerated. No grade 3/4 adverse events were seen. Three patients referred symptoms during rituximab infusion (grade 1) and all these reactions occurred in patients who did not receive premedication with steroids. Conclusions. Rituximab administration in a 90-minute infusion schedule is well tolerated and safe in this group of patients. This approach is beneficial, both in patients who are administered steroids and in patients who are not.

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RUTUXIMAB PLUS CLADOBINE OR CLADOBINE AND CYCLOPHOSPHAMIDE IN HEAVILY PRETREATED PATIENTS WITH INDIFFERENT LYMPHOPROLIFERATIVE DISORDERS

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Background. Preclinical studies have shown synergistic or additive effects of rituximab combined with purine nucleoside analogs, fludarabine or cladobine (2-CdA). Aim: In this report we present the results of our study evaluating the feasibility, efficacy and toxicity of the combined regimen consisting of rituximab plus 2-CdA (RC regimen) or rituximab, 2-CdA and cyclophosphamide (RCC) in the treatment of patients with heavily pretreated indolent lymphoid malignancies. Methods. Between March 2001 and November 2005 54 adult patients with relapsed or refractory low grade non-Hodgkin lymphoma (LG-NHL) and B-cell chronic lymphocytic leukemia (CLL) were treated according to RC/RCC regimen. The RC protocol consisted of rituximab at a dose of 375 mg/m² i.v. on day 1 and 2-CdA given at a dose of 0.12 mg/kg/d on days 2 to 6. In RCC protocol rituximab was administered at a dose of 375 mg/m² on day 1, 2-CdA 0.12 mg/m² days 2 to 4 and cyclophosphamide at a dose 650 mg/m² i.v. on days 2 to 4. The cycles were repeated every 28 days or longer if severe myelosuppression occurred. Guidelines for response were those developed by the NCI-sponsored Working Group. Results. Fifty four patients, 32 patients with B-CLL and 22 with LG-NHL entered the study and all of them were eligible. Thirty three patients (61.1%) were recurrent after prior therapy and 21 (38.9%) had refractory disease. All patients received 3 or more cycles of chemotherapy before RC/RCC treatment. Thirty-one patients were treated with RC regimen and 23 with RCC regimen. The RC/RCC courses were repeated at 4 week intervals or longer if severe myelosuppression occurred. One hundred fifty six cycles of RCC/RCC with median of 3 cycles per patient were administered (range 1-5 cycles). Six patients (11.1%), 2 with B-CLL and 4 with LG-NHL, achieved a complete response (CR). Thirty two patients (59.25%), including 23 with B-CLL and 9 with LG-NHL, had a partial response (PR). Overall response rate (OR) was 70.4% in the whole group, from 59.1% in LG-NHL to 78.1% in B-CLL patients. The median failure-free survival (FFS) of responders was 10.5 months. Hypersensitivity to RIT was the major toxicity of RC/RCC regimens and occurred in 14 patients (25.9%), mostly during the first infusion of RT. Severe neutropenia (grade III-IV) was seen in 9 patients (9.25%). Eight (14.8%) episodes of grade III-IV infections were observed. One patient died from severe pneumonia complicated with septic shock after second cycle of RCC regimen. Severe thrombocytopenia (grade III-IV) occurred in 4 patients (7.4%). Conclusion. RC and RCC regimens are highly effective and well tolerated modalities of treatment in heavily pre-treated patients with indolent lymphoproliferative disorders.

A RETROSPECTIVE STUDY TO ASSESS RELATIVE DOSE INTENSITIES IN PATIENTS WITH LYMPHOMA IN CENTRAL EUROPEAN COUNTRIES

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Background. Maintaining chemotherapy dose intensity is important for the successful treatment of cancer patients. However, neutropenia and its complications are major dose-limiting factors. Data on chemotherapy-related reductions in dose intensities for lymphoma patients from Central European (CE) countries remain sparse. Aim. To assess the relative dose intensities in patients with Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL) in CE countries. Methods. Chemotherapy treatment data from 1995 to 2004 were retrospectively collected from 484 patients undergoing chemotherapy treatment for lymphoma from 24 centres in 4 CE countries: Czech Republic (26%), Hungary (13%), Poland (44%) and Slovakia (17%). For this sub-analysis, 510 patients who received either doxorubicin, vinblastine, bleomycin and dacarbazine (ABVD) treatment for HL (117 patients) or cyclophosphamide, doxorubicin, vincristine and prednisone + rituximab every 21 days (CHOP21-R) for NHL (193 patients) were considered. Results. Of 116 HL patients with full data records (median age 29 years), 112 (96%) had classical disease and 4 (3%) had lymphocyte-predominant HL; for 189 NHL patients with full records (median age 56 years), 179 (95%) were B-cell and 10 (5%) were T-cell. ABVD was administered to 100% of the 117 HL patients selected for this study and CHOP21-R was administered to the 193 NHL patients, of which 59 patients (20%) also received rituximab. Dose delays > 7 days were observed in 271 out of 1583 cycles (17%; HL: 110 of 648-17%; NHL: 161 of 958 - 17%). Over all, 221 patients (72% of 306 considered for this analysis) experienced at least one dose delay during their treatment. This corresponds to 95 of 117 HL-ABVD patients and 126 of 189 NHL-CHOP21-R patients (i.e. 81% and 67% respectively). Among the hundred and forty-three patients (47% of 305 patients) experienced a dose reduction of ≥15% in at least one cycle, of which 90 patients (30% of 305) received ≥15% reduction in their overall dose. Dose reduction of ≥15% in any cycle occurred in 61 HL-ABVD patients (52% of 117) and in 82 NHL-CHOP21-R patients (44% of 188), with 51 HL-ABVD and 59 NHL-CHOP21-R patients receiving ≥15% reduction in their overall dose (44% and 21% respectively). The relative total dose intensity (RTDI, see Table 1) at the end of treatment was as follows: 55.6% of HL-ABVD patients received ≥85% RTDI and 59.3% received ≥90% RTDI; 73.4% of NHL-CHOP21-R patients received ≥85% RTDI and 59% received ≥90% RTDI. G-CSF was administered in 71 of 745 cycles (9.3%) chemotherapy and in 73 of 1124 cycles of NHL-CHOP21-R (6.5%). There were 48 unplanned hospitalisations in 50 patients (5 HD-AVBD and 25 NHL-CHOP21-R); 21 hospitalisations were neutropaenia-related. Summary/Conclusions. The reduction of RTDI, and its associated problems, in lymphoma patients receiving chemotherapy is a major concern. The data observed in CE countries are similar to US centres (Lyman et al. JCO 2004; 22: 4302-4311). Further analysis of these data will enable a better understanding of the implications of reduced RTDI in lymphoma and help to identify those patients who require preventative treatment.

Table 1. RTDI at end of treatment.

<table>
<thead>
<tr>
<th>Patient group</th>
<th>≤75%</th>
<th>≤75≤85% ≥2</th>
<th>≥85≤95% ≥95</th>
<th>≥90%</th>
</tr>
</thead>
<tbody>
<tr>
<td>HL-ABVD</td>
<td>32 (27.35)</td>
<td>20 (17.03)</td>
<td>38 (32.48)</td>
<td>27 (23.08)</td>
</tr>
<tr>
<td>NHL-CHOP21-R</td>
<td>33 (17.55)</td>
<td>7 (9.04)</td>
<td>69 (36.70)</td>
<td>69 (36.70)</td>
</tr>
</tbody>
</table>

*Total number of patients with data available for this analysis.

A COMPARISON OF 2-DEXT-2-[18F]FLUORO-D-GLUCOSE POSITRON EMISSION AND COMPUTER TOMOGRAPHY FOR STAGING OF PATIENTS WITH Hodgkin Lymphoma

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Background. Accurate staging in lymphoma patients (pts) has an important role in the treatment and allows miniization of toxic therapies, such as extended field radiation or overly aggressive chemotherapy. Particularly in HL a tailored therapy decrease the risk of secondary malignancies which exceeds 10% in several historical series in patients with early stage disease. Anatomic imaging modalities lack sensitivity and specificity because the definition of lymph node involvement is based on size criteria. During the last decade FDG-PET has been introduced for noninvasive staging of lymphoma. Methods. Herein we propose a prospective multicentric study with the aim to assess the impact of FDG-PET on the staging of pts with diagnosis of HL. A total of 186 consecutive pts coming from six Italian hematological Institutions underwent a FDG-PET scan in addition to conventional staging procedures, which include physical examination, laboratory data, bone marrow biopsy and imaging of the neck, thorax, abdomen and pelvis using CT scan. In general the adjunctive informations from PET did not influence the therapeutic options in use at a given centre at a particular time. Results. Pts characteristics were the following: 98 male and 88 female, 140 (75%) with diagnosis of nodular sclerosing classical HL, 28 (15%) mixed cellularity classical HL, 11 (6%) lymphocyte-rich classical HL, 2 (1%) lympho-
cyte-depleted classical HL and 5 (3%) non specified HL. At clinical and instrumental standard staging, 11 (6%) pts were stage I, 112 (60%) stage II, 42 (22%) stage III and 21 (12%) stage IV. FDG-PET and CT were concordant in 156 out 186 pts (84%). FDG-PET allowed to identify in 38 out 156 concordant stage more nodal (52 pts) or extranodal (6 pts: two bone, two spleen, two liver and spleen) involvement in comparison with CT imaging. In eight out 156 (5%) discordant stage CT showed one more involvement than FDG-PET in 64%, opposite in 100%, stage IV in 100% (bone marrow involvement in 86% and CNS involvement in 36%). Interna-
tional Prognostic Index (IPI) ≥ 3 in 71,4% of the cases, respectively. His-
tological analysis and immunohistochemistry performed on nodule tissue biopsy and/or bone marrow tumor cells showed a DLBCL in 9 and a ‘Burkitt-like’ in 4 patients, respectively. Cytogenetic analysis (conven-
tional cytogenetics and FISH analysis) showed in all cases the combina-
tion of t(14;18) translocation and c-myc rearrangement. All patients were treated with chemotherapy regimens (R-CHOP (n=8) or High-dose CHOP (n=6)), and could receive subsequent high-dose front-line ther-
apy with autologous (n=5) or allogeneic stem cell transplantation (n=2).
Most patients (12/18=66%) initially responded to induction chemother-
apy but disease response was dramatically short, precluding a planned stem cell transplantation in most cases. Despite salvage chemotherapy, all patients, even those who could receive early stem cell transplantation, progressively and the median overall survival from diagnosis is 4 months (1-10). In conclusion, DLBCL with a tandem t(14;18) translocation and c-myc rearrangement is a very aggressive entity with rapid progressive disease. Innovative strategies are warranted in this subgroup of patients.

MARKED ACTIVITY OF BORTEZOMIB, RITUXIMAB, AND DEXAMETHASON (BORID) IN HEAVILY PRETREATED PATIENTS WITH MANTLE CELL LYMPHOMA

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Background. Bortezomib (B) belongs to a new class of anti-cancer agents, the proteasome inhibitors, and has documented activity in multiple myeloma and mantle cell lymphoma (MCL). Preliminary studies suggest that B has synergistic activity with rituximab (R), which provides support for the treatment of patients with relapsed or refractory non-Hodgkin’s lymphoma (NHL), but relapse rates remain high, especially in those with mantle cell lymphoma (MCL). Aims. We have initiated a phase I/II study in relapsed/chemotherapy refractory MCL to evaluate the activity and safety of B in combination with R and dexamethasone (BORID). Patients and Methods. A treatment cycle consists of B at 1.3 mg/m² administered on days 1, 4, 8, and 11, R at 375 mg/m² on day 1, and dexamethasone 40 mg orally on days 1 to 4. Cycles are repeated every 3 weeks for a total of 6 treatment cycles. Patients (pts) with progressive MCL after at least one prior line of therapy (including CHOP or a CHOP-like regimen) are eligible. Results. Up to now, we have enrolled 11 pts (median age, 67 years; range, 40 to 75 years) after a medi-
an of 3 lines of prior therapies (range, 1 to 6) including R in 9 pts, high-
dose therapy in 4 pts, and thalidomide in 5 pts. Median time between start of frontline therapy and study inclusion was 42 months (range, 11 to 96 months). Severe adverse events (> grade II) included infections (herpes zoster in 2 pts, bacterial pneumonia, mucosal candidiasis), peripheral neuropathy (3 pts), fatigue (2 pts) and vasculitic skin infiltr-
tates in 3 pts. Thrombocytopenia (< 50 g/L) occurred in 2 pts. All adverse events were manageable by standard means of supportive care and pro-
longation of the treatment interval between cycles. Of 9 pts evaluable for efficacy, 8 have achieved a response (3 CR, 4 PR), and 1 pt experi-
ced stable disease. Pts in CR were also negative for disease activity by PET scanning. Skin infiltrates (histologically proven T-cell infiltrates) preceded achievement of CR in 2 pts. Among 7 pts with follow-up beyond 6 months, 2 pts have relapsed (progression-free survival 9 and 11 months, respectively), and 5 pts are still progression-free at 12, 11, 7, and 6 months, respectively, after treatment initiation. Recruitment of patients is ongoing, and updated results will be presented. Conclusions. Data obtained to date far indicate that BORID has promising activity and manageable toxicity in patients with heavily pretreated MCL, and develop-
ment of a vesiculat rash may be an early indicator of a favorable response.

CLINICAL AND CYTOGENETIC CHARACTERISTICS OF HIGH-GRADE NON-HODGKIN LYMPHOMA (HG-NHL) WITH A COMBINATION OF T(14;18) TRANSLLOCATION AND C-MYC REARRANGEMENT: A VERY AGGRESSIVE ENTITY WITH A DISTINGUISH PROGNOSIS


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Diffuse Large B cell lymphoma (DLBCL) is an heterogeneous entity with various clinical, cytological, cytogenetic and molecular features. In this single center lymphomatoepigenetic analysis, we report the results of a cohort of 14 patients treated from 1997 to 2005 with a diagnostic of DLBCL or ‘Burkitt-like’ lymphoma characterized by a tandem t(14;18) translocation and c-myc rearrangement. Patients were 9 males, 5 females with a medi-
an age of 52 years (36-75). At the time of diagnosis, all patients presented

with poor clinical and biological features: B symptoms in 78.5%, ESR > 50 mm in 85.7%, elevated LDH in 100%, stage IV in 100% (bone

marrow involvement in 86% and CNS involvement in 36%). Interna-
tional Prognostic Index (IPI) ≥ 3 in 71.4% of the cases, respectively. His-
tological analysis and immunohistochemistry performed on nodule tissue biopsy and/or bone marrow tumor cells showed a DLBCL in 9 and a ‘Burkitt-like’ in 4 patients, respectively. Cytogenetic analysis (conven-
tional cytogenetics and FISH analysis) showed in all cases the combina-
tion of t(14;18) translocation and c-myc rearrangement. All patients were treated with chemotherapy regimens (R-CHOP (n=8) or High-dose CHOP (n=6)), and could receive subsequent high-dose front-line ther-
apy with autologous (n=5) or allogeneic stem cell transplantation (n=2).
Most patients (12/18=66%) initially responded to induction chemother-
apy but disease response was dramatically short, precluding a planned stem cell transplantation in most cases. Despite salvage chemotherapy, all patients, even those who could receive early stem cell transplantation, progressively and the median overall survival from diagnosis is 4 months (1-10). In conclusion, DLBCL with a tandem t(14;18) translocation and c-myc rearrangement is a very aggressive entity with rapid progressive disease. Innovative strategies are warranted in this subgroup of patients.
90Y ibritumomab tiuxetan did not correlate with the risk of relapse as determined by univariate analysis. Summary/Conclusions. 90Y ibritumomab tiuxetan may be safely incorporated into conditioning regimens prior to ASCT, even in patients >60 years. Late relapses were uncommon, which suggests this approach may lead to durable remissions in MCL.

**0210**

**GASTRECTOMY PLUS CHEMOTHERAPY VS. CHEMOTHERAPY ALONE IN GASTRIC DIFFUSE LARGE B-CELL LYMPHOMA OF EARLY STAGE**

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**Background.** Stomach represents the most common site of primary extranodal diffuse large B-cell lymphoma (DLBCL). The ideal therapeutic strategy in gastric DLBCL remains controversial. Gastrectomy prior to chemotherapy is favored by some, while others prefer sole administration of chemotherapy. Aim: To evaluate and compare these two therapeutic strategies, gastrectomy plus chemotherapy and chemotherapy alone, in gastric DLBCL of early stage in the context of a retrospective study. Methods: Between 1979 and 2003, 78 patients with gastric DLBCL of early stage (I-II, no X) were diagnosed and treated in our department. Patients were divided in group A that comprised 46 (59%) patients, who underwent total gastrectomy prior to chemotherapy and group B that consisted of 32 (41%) patients, who received chemotherapy alone. Chemotherapy in both groups included CHOP and CHOP-like regimens. Median number of chemotherapy cycles administered in groups A and B was 6 (5-9) and 6 (2-9) respectively (p < 0.05). Rituximab was also administered in 14 (30.4%) patients of group A and in 12 (37.5%) patients of group B (p > 0.05). Five (11%) patients of group A and 2 (6.3%) of group B received additionally radiation therapy (p > 0.05). The characteristics of our patients (gender, age, stage, IPI, presence of B symptoms and extranodal involvement other than primary), as well as response rates, were compared between the two groups using chi-square tests. Disease-free survival (DFS), overall survival (OS) and failure-free survival (FFS) were estimated according to the Kaplan-Meier method. Differences in survival rates were assessed using the log-rank test. Results: Median follow-up time for patients in groups A and B was 70 (2-270) and 46 (2-155) months respectively. On an intention-to-treat basis, the complete response rate was 91.3% for group A and 87.5% for group B (p > 0.05). DFS, OS and FFS rates at 4 years in groups A and B were 89.1% and 92.9%, 80.2% and 81.5%, 75.3% and 76.3% respectively (p > 0.05). Conclusion. Gastrectomy plus chemotherapy failed to prove its superiority as treatment for gastric DLBCL of early stage in our study. Similar response and survival rates were achieved with chemotherapy alone, saving at the same time the patient from the morbidity impact of gastrectomy on quality of life.

**0211**

**PREDICTORS OF SURVIVAL IN ELDERLY PATIENTS WITH AGGRESSIVE LYMPHOMA. A LONG-TERM FOLLOW-UP OF A RANDOMISED TRIAL**

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**Background.** We previously reported the results of a study in elderly patients (>60 years) with aggressive non-Hodgkin lymphoma (NHL) randomizing patients to receive CHOP (doxorubicin 50 mg/m²) or CNOP (mitoxantrone 10 mg/m²) with or without G-CSF (5 µg/kg from day 2 until day 10-14 of each cycle every 3 weeks; 8 cycles; Blood 2003;101: 3840). In that analysis 85% of patients were alive after a median follow-up time of 57 months. The main findings were: 1) patients receiving CHOP fared better than those given CNOP chemotherapy and 2) the addition of G-CSF reduced the incidence of severe granulocytopenia and infections. We now report long-term follow-up data with special emphasis on predictors of survival. Methods. The study included 455 previously untreated patients (median age 71 years; range 60-86 years) with stage II to IV aggressive NHL. Forty-seven patients previously hospitalized for class I to II congestive heart failure were randomized to receive CNOP with or without G-CSF (not included in the CHOP versus CNOP analysis). Results. After a median follow-up time of 115 months (18-151 months) 19% (88/455) of patients were alive. In univariate analysis CNOP treatment (p < 0.001; figure), increasing age (p < 0.001), poor performance status (p < 0.001), high LDH (p < 0.001), advanced stage (p < 0.05), and the presence of more than one extranodal disease manifestation (p = 0.045) negatively influenced overall survival from diagnosis. Gender (p = 0.156), presence of B symptoms (p = 0.079), bulky disease (p = 0.085), and treatment with G-CSF (p = 0.094) did not significantly affect overall survival. In multivariate analysis, all factors significant in univariate analysis except extranodal disease, remained significant and independent predictors of survival. Conclusion. At long-term follow-up of this large multicenter randomised study of elderly with aggressive NHL the projected 10-year survival of CHOP treated patients was in excess of 20%. Increasing age, poor performance status, high LDH and advanced stage independently predicted a poor survival.

**Figure 1. Survival according to type of chemotherapy.**

**0212**

**UPDATE REPORT ON 78 PATIENTS WITH POST TRANSPLANT LYMOPHOPROLIFERATIVE DISORDERS FOLLOWED BY A SINGLE CENTER**

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**Background.** Post transplant lymphoproliferative disorders (PTLDs) are a well recognized complication after solid organ transplantation, related to the chronic immunosuppressive regimen. The wide spectrum of histological features, clinical variability and high therapy-related toxicity make management of PTLD patients difficult. Aims. This abstract provides an update of clinical and pathological data of PTLD patients, followed at our Center between 1989 and 2006. Methods. Our study included 78 patients with a diagnosis of PTLD in solid organ transplant recipients (36 heart, 23 liver, 17 kidney and 2 lung). Morphological classification was made according to WHO criteria. In 72/78 patients tumour EBV status was tested by in situ hybridization for EBV encoded RNA (EBER). Results. It was not possible to assign 2/78 cases to any histological category because of inadequate specimens; 6/76 (8%) evaluable patients were classified as having Plasmacytic Hyperplasia (PH), 13/76 (17%) Polymorphic Lymphoproliferative Disorders (PLD) and 57/76 (75%) Malignant Lymphoma (ML). Fifty-nine of seventy-eight (75%) patients developed late onset PTLD (> 12 months from transplant). Among patients tested for EBER, 49/72 (68%) were EBV positive. While EBER positive PTLDs were heterogeneous with regard to time of occurrence and histological characteristics (6 PH, 11 PLD, 31 ML, 1 not classified), all EBER negative PTLDs were late onset ML. Diagnosis was obtained post-mortem in 9/78 patients. The treatment was tailored according to clinico-pathological features; 52/69 (75%) patients received a single agent or a combined regimen of chemotherapy, associated with antiviral drugs in EBER positive forms. Rituximab has been introduced in the therapeutic schedule of CD20+ PTLDs since 2000. It was administered to 24 patients, combined with chemotherapy in all but 2 cases.
Radiation and surgery were used when indicated. Eleven patients died early, before any treatment was completed (median time 18 days, range 5-56); 2/69 patients were lost at follow-up. Therefore, a total of 56 patients underwent their scheduled treatment and were evaluable for the outcome. We observed 10/56 deaths because of treatment related toxicity or disease progression. Complete remission (CR) was achieved in 45/56 (80%) patients. Of these, 8 (15%) relapsed, mostly responsive to second line therapy (7/8 patients), and 10 patients died because of late treatment-related toxicity or infection. One patient is still alive in partial remission (PR) at 23 months. The median survival time of evaluable patients was not reached at 3250 days (see Figure 1).

**Background.** We studied the prevalence of second cancer in nongastric MZL of MALT in a population-based study from Northern Italy. Marginal zone B-cell lymphomas (MZL) of MALT show a peculiar relationship with the triad autoimmunity-infection-immunosuppression. For this reason, these lymphomas have been studied for the risk of second cancers. Most series reported so far regard patients with gastric MALToma, while data on nongastric MZL of MALT are lacking.

**Aims.** To define the risk of second cancer in nongastric MZL of MALT in a population-based study from Northern Italy.

**Methods.** We studied the prevalence of second cancers in a series of 157 patients with nongastric MZL of MALT consecutively diagnosed in two haematological Institutions of the Northern Italy region Lombardia. We compared the occurrence of second cancer with respect to the general population by calculating the standardized incidence ratio (SIR), with the age- and sex-specific incidence rates of the Cancer Registry of Lombardia as a reference.

**Results.** A history of 30 additional neoplasms was documented in 29 patients (18%) (18 females and 11 males): 21 previous, 3 concurrent, and 6 subsequent. The malignancies were: 25 solid tumors, 2 hematological diseases (1 Hodgkin’s lymphoma and 1 essential thrombocythemia), 3 non-melanoma in situ skin cancers. One patient had two malignancies (breast cancer and essential thrombocythemia), both prior to the diagnosis of cutaneous lymphoma. The sites of solid cancers were: 8 breast, 4 endometrium, 4 skin, 3 thyroid, 2 lung, 1 prostate, 1 colon, 1 small intestine, 1 salivary gland, 1 bladder, 1 ovary and 1 stomach. In 4 patients the site of cancer and lymphoma was the same. For the entire group, the SIR of an additional malignancy was 0.8 (95% CI: 0.55-1.17, p=0.2). The relative rate of an additional malignancy was 0.7 for males (95% CI: 0.39-1.26, p=0.2) and 0.89 for females (95% CI: 0.55-1.46, p=0.6). The comparison of risks between males and females was not significant (SIR ratio 1.28, 95% CI: 0.59-2.76, p=0.5).

After excluding non-melanoma skin cancers, the SIR of a second tumor was 0.75 (95% CI: 0.5-1.12, p=0.2). The relative rate of a second tumor was 0.6 for males (95% CI: 0.31-1.15, p=0.1) and 0.89 for females (95% CI: 0.54-1.47, p=0.6). The comparison of risks between males and females was not significant (SIR ratio 1.49, 95% CI: 0.65-3.4, p=0.5).

After excluding all previous malignancies, the SIR of a second cancer was 1.52 (95% CI: 0.69-2.55, p=0.4). All concomitant and subsequent malignancies were invasive tumors. The relative rate of a second cancer was 1.46 for males (95% CI: 0.61-2.51, p=0.4) and 1.19 for females (95% CI: 0.44-3.16, p=0.7). The comparison of risks between males and females was not significant (SIR ratio 0.81, 95% CI: 0.22-3.02, p=0.8).

**Conclusions.** These data demonstrate that patients with nongastric MZL of MALT are not at increased risk for second cancer compared to the general population of the same geographical area. However, since nongastric MALT lymphoma is a long-lasting disease of advanced age with high risk of relapse, a careful clinical follow up is always warranted.

**0214 ACHIEVEMENT OF MOLECULAR REMISSION AFTER FIRST LINE TREATMENT PROLONGS SURVIVAL IN FOLLICULAR LYMPHOMA PATIENTS**

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**Background.** However is follicular lymphoma (FL) still considered conventionally incurable disease, prolonged complete remissions were reported. Results of recent studies suggest, that patients who achieve complete remission (CR) with PCR bcl-2/IgH negativity (molecular remission, CRm) have better long term outcome. Aims. To evaluate whether achieving of molecular remission after first line treatment have an impact on disease free (DFS) and overall (OS) survival in all risk subgroups, previously untreated, follicular lymphoma patients. Methods. 104 (pts) with FL were diagnosed and treated in our department during last 8 years. All of them were examined with a qualitative PCR (bcl-2/IgH) from bone marrow (BM). Bcl-2/IgH (MBr, mcr or long-distance PCR) positivity was observed in 55 pts (57%) at the time of diagnosis. 91% of bcl-2/IgH+ pts had an advanced disease stage (III/IV), BM involvement was present in 43.5%, bcl-2/IgH+ pts. First line treatment was stratified acording generally used risk factors (FLIPI, GELF, β-2-m level), bulk disease. Patients under 60 (65) y.o. with high risk disease (FLIP ≥3 or additional risk factors) were indicated to stem cell transplantation (SCT). 19 patients underwent autologous and 1 patient allogenic SCT. 19 patients underwent autologous and 1 patient alloigeneric SCT. 56 patients were treated conventionally (CHOP or fludarabine based regimens). Rituximab was administered as first line concomitant chemo-immunotherapy in 21 pts (equally in both groups). PCR (BM and/or peripheral blood) was reevaluated on the day +100 after SCT or at the point of restaging and during follow-up every 6 months.

**Figure 1. Event free survival: impact of residual disease.**
Conclusions. We investigated efficacy and safety of adding Rituximab (R) to induction and intensified HDC as part of first line treatment in pts with aa-IPI at Intermediate-High (IH) or High (H) risk with B-DLCL and compared two groups of pts enrolled in a randomized phase II clinical trials with up-front HDC and ASCT with or without R. Aims and Methods. 118 previously untreated pts <61 years with B-DLCL, stage III-IV at aa-IPI IH or H risk were treated: 41 pts were enrolled into HDC trial (control group; August 1991-August 1995) and 77 pts into R-HDC trial (study group; January 2001-December 2004). Treatment in R-HDC study group consisted in an induction treatment lasting two months with four courses of R-MegaCEOP chemotherapy (R 375 mg/m^2 day1, CTX 1200 mg/m^2 + EPI 110 mg/m^2 + VCR 1.4 mg/m^2 day8 and 5FU 40 mg/m^2 days5-7) every 14 days with G-CSF support; then two courses of intensified chemoimmunotherapy R-MAD (Mitoxantrone 8 mg/m^2 + ARAC 2000 mg/m^2/day 12+/Dexamethasone 4 mg/m^2/12h for 3 days and R 375 mg/m^2 day4 and before PBSC harvest) followed by ASCT with BEAM as conditioning regimen. Treatment in HDC control group was an induction treatment lasting two months with MACOPB x 8 weekly infusions followed by the same conditioning regimen and HDC (Gem and HDT; BEAM and ASCT).

IF RT was given to areas of previous bulky disease in both trials. Results. Pts characteristics in both trials were comparable with no statistically significant differences: median age was 45 years (19-60); 51% were at H risk; 36% had bone marrow (BM) involvement, 80% LDH>normal and 42% extranodal sites.1 Complete Response at the end of the treatment was: 60 pts (78%) in R-HDC group and 28 (68%) in HDC group (p=0.25). Failures (17% vs 25%) and toxic deaths (5% vs 7%) were comparable between the two groups (R-HDC vs HDC). Short-term toxicity appeared similar. NO MDS or ANLL or solid tumours were reported in both arms. No differences were observed in neutrophils >500/mm^3 and platelets >50000/mm^3 engraftment; median times in R-HDC vs HDC were: 9 vs 10 and 15 vs 16 days. Median follow-up was 36 months in study group and 72 months in control group. Three-year failure-free survival (FFS) and 3-yr overall survival (OS) rates in R-HDC group vs HDC group were: FFS 64% vs 46% (p=0.016); OS 80% vs 54% (p=0.004). Improved outcome for pts treated with R-HDC was confirmed in both pts groups (IH and H risk). A Cox’s model was performed to adjust the effect of treatment for competing risk factors (age, IPI, BM involvement, number of extranodal sites). In this multivariate analysis the risk of failure and death was confirmed as significantly reduced in R-HDC group: adjusted hazard ratio (R-HDC vs HDC) was 0.56 (95% CI=0.30-1.01, p=0.05) for FFS and 0.42 (95% CI=0.21-0.88, p=0.02) for OS. Conclusions. These results suggest that the addition of Rituximab to induction and intensified chemotherapy before BEAM and ASCT is effective and safe in B-DLCL at poor prognosis.
cation to HDT (induction vs salvage therapy) and type of salvage therapy as well as etoposide combined with corticosteroid (Eto-CS). The mean expression of the mRNA of the hMask and Mask-BF3ARF genes were 3 and 4 times increased, respectively, compared with control. Quantification of hMask mRNA with a poly(A+) signal and it is a new splice variant of Mask. In Drosophila, MASK protein seems to interact with members of the Receptor Tyrosine Kinase (RTK) signalling pathway and loss of this interaction increases programmed cell death, reduces cell proliferation, inhibits photoreceptor differentiation, affects RPE cell-dependent processes but does not affect MAPK (Mitogen Activated Protein Kinase) activation. However, the biological functions of these proteins in humans remain still unknown.

The major role of Mask in this subset of pts could be in the improvement of salvage therapy results in order to increase the number of pts who are able to undergo HDT and ASCT.

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**0218**

COMPARATIVE ANALYSIS OF TREATMENT OUTCOMES WITH CHOP REGIMEN, ETOPOSIDE PLUS CORTICOSTEROID AND PREDNISOLONE IN ADULT PATIENTS WITH HEMOPHO- CYTIC LYMPHOCYTOSIS/IC: BASED ON UNDERLYING DISEASES

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**Background.** The outcome of CHOP treatment in the case of lymphoma-associated hemophagocytic lymphohistiocytosis (HLALH) and EBV-associated HLH (EBV-HLH) has rarely been reported. **Aims.** The present study analyzed the treatment outcomes for CHOP chemotherapy as well as etoposide combined with corticosteroid (Eto-CS) and prednisolone (PRS) in adult patients with EBV-HLH and LAHLH. **Methods.** 46 adult patients older than 16 years of age were diagnosed with HLH. Among these patients, 30 treated with CHOP chemotherapy (n=18), Eto-CS (n=6), and PRS (n=6) were reviewed retrospectively. **Results.** With CHOP chemotherapy, complete remission (CR) was achieved in 5/18 patients (27.8%), partial remission (PR) in 5/18 (27.8%), and the overall response rate was 55.6%. With Eto-CS therapy, PR was achieved in 5/6 patients (50%), however CR was achieved with PRS therapy. CR was achieved in 1/6 patients (16.7%) and PR in 1/6 (16.7%). The median response duration (RD) was not reached and the 3-year estimated RD was 68.57% for the CHOP chemotherapy, while the median RD was three weeks for the Eto-CS therapy and one week for the PRS therapy, with a median follow-up of 132 weeks. The median duration for the overall survival (OS) was 16 weeks and the 3-year estimated OS rate 40.65% for the patients treated with CHOP therapy, yet only four and two weeks for the patients treated with Eto-CS and PRS, respectively (p=0.016). Conclusions. CHOP chemotherapy seemed to be useful in adult patients with LAHLH and EBV-HLH. Additional treatment including stem cell transplantation may also be needed, especially for patients with poor prognostic factors.

**0219**

PP2500 mRNA, A SPlice Variant of the Multiple AnKinin Repeat Single KH Domain (Mask), is Highly Expressed in Plasma Cells of Multiple Myeloma


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**Background.** The Ankyrin (ANK)-repeat is one of the most common protein sequence motifs, which leads itself to variation in overall domain size by simple sequence duplication or deletion. The Mask (Multiple Ankyrin Repeats Single KH domain) gene, which codifies an ANK-repeat protein, is located in chromosome 5(q31.3) and it is composed of 39 exons. It generates isoforms by alternative splicing. The first splice variant (hMask) lacks the 10A exon of the Mask gene, generating a mRNA containing 34 exons. The other, Mask-BF3ARF, results from fusion of splice variant hMask, with the two last exons of the gene Eif4ebp5 (exons B and C) and an intermediate exon (exon 0), generating 36 exons. Recently, a new splice variant, denominated PP2500, was deposited in the data base Genebank, it presents the first 10 exons, homologous to Mask mRNA with a poly(A) signal and it is a new splice variant of Mask. In Drosophila, MASK protein seems to interact with members of the Receptor Tyrosine Kinase (RTK) signalling pathway and loss of this interaction increases programmed cell death, reduces cell proliferation, inhibits photoreceptor differentiation, affects RPE cell-dependent processes but does not affect MAPK (Mitogen Activated Protein Kinase) activation. However, the biological functions of these proteins in humans remain still unknown.

**Conclusion.** The role of Mask in this subset of pts could be in the improvement of salvage therapy results in order to increase the number of pts who are able to undergo HDT and ASCT.

**0220**

A PHASE II STUDY OF THALIDOMIDE, DEXAMETHASONE AND PEgylated Lyposomal DOxorubicin (THADD) FOR UNtREATED PATIENTS WITH MULTIPLE MYELOMA AGED OVER 65 YEARS

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**Background.** No standard therapy have been yet identified for elderly patients with multiple myeloma (MM) despite two thirds of cases affected by this incurable malignancy are older than 65 years. The combination melphalan-prednisone yields unsatisfactory results and high-dose therapy, despite feasible also in elderly patients, can be an unavailable option because of pre-existing medical comorbidities. Improvements in the outcome of elderly MM patients have been obtained using thalidomide as single agent or in combination with dexamethasone or conven-
tional chemotherapy. Aims. We report the results of a phase II study including 50 newly diagnosed patients with symptomatic MM older than 65 years regardless of comorbidities, performance status and renal function. Methods. All patients received thalidomide 100 mg/day continuously, pegylated liposomal doxorubicin 40 mg/m² on day 1 every 28 days, dexamethasone 40 mg on days 1-4 and 9-12 (ThaDD). They also were given warfarin 1.25 mg/day as antithrombotic prophylaxis and ciprofloxacin 250 mg twice daily after a high incidence of infections was recognized. Median age was 71.5 years (range 65-78) and 64% were older than 70 years. Thirty-nine patients (78%) had clinical stage III, 37 (74%) ISS’2 and 7 patients (14%) a serum creatinine level > 2 mg/dL. Moreover, unfavourable cytogenetics were detected in 85% of patients with a median value of 7. A cut-off value of 7% after ciprofloxacin has been added to the protocol. Grade 3-4 nonhematological side effects were mainly attributable to thalidomide and consisted of constipation (4%), fatigue (6%) and tremors (4%). Regarding toxicity due to pegylated liposomal doxorubicin, 2 patients experienced grade 3-4 mucositis and one grade 3 palmar-plantar erythrodysesthesia. Venous thromboembolic events occurred in 7 patients (14%) but only one patient experienced pulmonary embolism. Conclusions. Our study demonstrates that the combination low-dose thalidomide, pegylated liposomal doxorubicin and high-dose dexamethasone is very effective in the treatment of elderly patients with MM as it induces an ORR and particularly a CR rate higher than those reported with all other thalidomide-based regimens. It results well tolerated also by oldest fragile patients and thrombotic as well as infectious complications can be prevented by adequate prophylaxis.

0221

MONOKINE-INDUCED BY INTERFERON-γ SERUM LEVELS ARE A MARKER OF DISEASE LOAD AND CORRELATE WITH PROGNOSIS IN MULTIPLE MYELOMA

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Background. Monokine-induced by interferon-γ (MIG) is a chemokine known to be produced by monocytes and macrophages in response to interferon-γ, and acts as a chemoattractant to T-lymphocytes and other inflammatory cells. Besides its role in the host immune response to infections and in neoplastic disease, MIG has also been implicated as chemokine acting in an autocrine loop to stimulate tumor cells through its receptor CXCR3. Myeloma cells are known to express CXCR3 (Pellegrino et al., 2004), however it is unclear if MIG is of biological significance in myeloma in vivo. Aims. We have shown recently that multiple myeloma oncogene 1 (MUM1) expression in myeloma cells correlates with prognosis in this disease (Heintel et al., 2005), and MUM1 is known to upregulate MIG gene expression in B cell malignancies (Urashini et al., 2005). This led us to evaluate the potential prognostic significance of MIG serum levels in a series of myeloma patients. Methods. MIG serum levels were determined by a commercially available ELISA (R&D Systems) in a series of 54 newly diagnosed myeloma patients. Serum from 5 healthy volunteers, 4 patients with osteoporosis, and 4 patients with chronic obstructive pulmonary disease (COPD) were used as controls. Results. Median MIG serum level was 32.3 pg/ml in healthy volunteers, range 22.7-52.79 pg/ml. In patients with osteoporosis and COPD the levels were higher (median 133.0, range 47.2-202.3 pg/ml) and median 54.9, range 28.52-200.2 pg/ml, respectively. In 54 newly diagnosed myeloma patients the median MIG level was 219.8, range 27.6-1966.0 pg/ml. When myeloma patients were stratified according to a MIG level < 200 and MIG > 200, a highly significant survival difference for the 2 cohorts was observed. While median survival was 88.2 months for patients with MIG < 200, patients with high MIG serum levels (> 200) had a survival of only 17.0 months (p=0.00409; see Figure). Serum-MIG levels correlated with markers of disease burden, including β2-microglobulin levels and extent of bone marrow plasma cell infiltration. MIG showed a negative correlation with hemoglobin and albumin levels. Interestingly, no correlation was found with C-reactive protein levels, indicating that MIG is not associated with an inflammatory response in myeloma. Preliminary experiments show that MIG mRNA is expressed in 1 out of 4 myeloma cell lines, with upregulation of expression seen after stimulation with interferon-γ in the positive line, but not in those without baseline MIG expression. Summary/Conclusions. MIG serum levels correlate with markers of disease burden in myeloma and high MIG levels are associated with a poor outcome in this disease.

0222

VAD-DOXIL VS. VAD-DOXIL PLUS THALIDOMIDE AS INITIAL TREATMENT IN MYELOMA PATIENTS: INTERIM ANALYSIS OF A MULTICENTER RANDOMIZED TRIAL OF THE GREEK MYELOMA STUDY GROUP

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Background. VAD-doxil and VAD-doxil plus thalidomide have already been separately evaluated, as initial cytoreductive treatment in multiple myeloma, in two previous clinical trials of our study group. Both regimens proved effective yielding overall (complete and partial) response rates of 61.3% and 74% respectively, while toxicity remained acceptable in both studies. Aims. To compare the efficacy and toxicity of these two regimens in the context of a multicenter randomized clinical trial. Results of an interim analysis are presently reported. Methods. Patients randomized in arm A received vincristine 2 mg IV, liposomal doxorubicin 40 mg/m² IV in a single dose on day 1, and dexamethasone 40 mg PO daily for 4 days. The regimen was repeated every 4 weeks. Dexamethasone was also administered on days 15-18 of the first cycle. Patients randomized in arm B received additionally thalidomide 200 mg PO daily at bed-time. Response to treatment was the primary objective of the study and was evaluated after the completion of 4 cycles. Subsequently, patients were allowed to proceed to high dose chemotherapy or to receive two additional cycles of the same regimen. Response and toxicity were evaluated according to EBMT and NCI criteria respectively. Patients’ characteristics, response and toxicity rates were compared using two-independent- samples tests and x² tests. Results. Between June 2002 and December 2005, 230 patients entered the study, 115 randomized in each arm. To date, 196 patients are evaluable for toxicity and 160, 80 in each arm, for efficacy. The two treatment groups were well-balanced regarding the usual prognostic characteristics. On an intention-to-treat basis, overall response rate was 66.3% and 81.3% in arms A and B respectively (p=0.045). Neutropenia, thrombocytopenia, infections, mucositis, palmar-plantar erythrodysesthesia, deep venous thrombosis and early mortality were not significantly different (p>0.05) between arms A and B.
**Bortezomib Demonstrates Superior Survival Compared with High-Dose Dexamethasone and Higher Response Rates After Extended Follow-Up in the Apex Trial in Relapsed Multiple Myeloma**

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**Background.** In the open-label, international, multicenter phase 3 APEX trial, 669 patients with relapsed multiple myeloma (MM) following 1-3 prior therapies were randomized to receive bortezomib (VELCADE®) or dexamethasone (Dex). Patients with progressive disease on Dex were eligible to cross over to bortezomib. Patients receiving bortezomib achieved significant improvement in survival, time to progression (TTP), and response rate (CR + PR, EBMT criteria). Consequently, the Dex arm was halted early and all patients receiving Dex were allowed to cross over to bortezomib. **Aims.** To update survival data for both the bortezomib and Dex arms of the APEX trial, and to update efficacy data for the bortezomib arm. **Methods.** Updated overall and 1-year survival rates were analyzed for both arms based on median follow-up of 22 months in surviving patients and deaths in 44% of patients. Updated response rate, time to response (TTR), duration of response (DOR), TTP were analyzed for both arms. Matched-pairs analyses were performed to compare survival and response rate in patients receiving bortezomib earlier (bortezomib arm) or later (Dex arm patients who crossed over to bortezomib arm). **Results.** Patients received a median of 6 cycles of bortezomib. Median survival was 29.8 months vs 23.7 months (p=0.0272), and 1-year survival rate was 80% vs 67% (p=0.0002), for bortezomib vs Dex, despite >62% of Dex patients crossing over to bortezomib. With extended follow-up, overall response rate by EBMT criteria with bortezomib improved from 45% in the initial analysis to 48% (+3%), and 56% of responders achieving an improved response after cycle 2, and 54% of responders achieving first response after cycle 2. The proportion of patients achieving maximum M-protein reduction continues to increase over the entire course of study-specified treatment (up to 8 cycles). Median TTP (6.2 months), TTR (1.4 months), and DOR (7.8 months) with bortezomib were unchanged compared with initial analysis. Median DOR was 11.5 months in patients with 100% M-protein reduction, and 7.6 months in patients with >50% but <100% M-protein reduction. Overall response rate and median survival in patients receiving bortezomib earlier vs later were: 44% vs 34%, and not reached vs 16.4 months, respectively (Table: Conclusions). After extended follow-up, significantly longer survival with bortezomib compared with Dex was confirmed, despite substantial crossover from Dex to bortezomib. Response rates are higher, with many patients achieving best responses after longer duration of therapy. Patients achieving 100% M-protein reduction tended to have a longer DOR. Additionally, patients receiving bortezomib earlier appear to have a higher response rate and longer survival.

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**Table: Conclusions**

<table>
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<tr>
<th>Bortezomib earlier</th>
<th>Bortezomib later</th>
</tr>
</thead>
<tbody>
<tr>
<td>Response rate (%)</td>
<td>44 vs 34</td>
</tr>
<tr>
<td>CR</td>
<td>9 vs 7</td>
</tr>
<tr>
<td>PR</td>
<td>35 vs 27</td>
</tr>
<tr>
<td>near CR</td>
<td>6 vs 10</td>
</tr>
<tr>
<td>Median survival, months</td>
<td>Not reached*</td>
</tr>
</tbody>
</table>

*Hazard ratio = 0.75; p=0.1722
Background. Autologous stem cell transplantation (ASCT) has an established role in the treatment of symptomatic multiple myeloma (MM). Reliable and simple staging of MM is important for accurate prognostic evaluation and for the comparison of data from different clinical trials. Attempts to improve the widely accepted Durie-Salmon (DS) staging system have led to the development of numerous new prognostic systems, that have not been universally accepted. Recently a new International Staging System (ISS) was presented. It has shown promise in patients (pts) treated by conventional as well as high-dose chemotherapy and is based on a simple combination of serum β2microglobulin (β2M) and albumin (ALB) values (stage 1 = β2M under 5.5 mg/L and ALB above 3.5 g/dL; stage 2 = β2M under 5.5 mg/L and ALB under 3.5 g/dL, or β2M from 3.5 mg/L to 5.5 mg/L; stage 3 = β2M above 5.5 mg/L). Aims. The status of disease parameters for better survival of MM pts after transplant are: age under 60 years (p<0.001), IgA typ of monoclonal immunoglobulin (p=0.036), renal impairment (p=0.007), no achievement of CR after ASCT (p< 0.001). The status of disease according to ISS, age, clinical response after transplant, type of therapies and specific strategies to overcome therapy-resistance will only be possible if we are able to recognize and understand the biological subtypes of myeloma. Therefore, there is a continuous need to improve the molecular classification of MM. Aims. Our hypothesis is that unsupervised cluster analysis of gene expression profiles will provide an improved molecular classification of myeloma patients compared to cytogenetics alone. The aim of this study is to test this hypothesis. Methods. Expression data of 173 newly diagnosed, untreated myeloma patients previously reported by Tian et al. (NEJM 2003,349:2483-2494) were downloaded from the Gene Expression Omnibus (www.ncbi.nlm.nih.gov/geo), accession number GDS531. The dataset contains expression data that were obtained using Affymetrix U95Av2 arrays and were normalized using the method of global scaling, provided in the Affymetrix MAS5.0 software. Unsupervised hierarchical cluster analysis was performed with complete linkage and Euclidean distance as similarity metric, using the Omnimix package. Supervised analyses were performed with the use of SAM software. Cluster-specific gene signatures were identified based on available annotations in Gene Ontology and the GenMAPP database. We have retrospectively evaluated 181 pts with MM undergoing autologous transplant (ASCT) in our centre between 1995-2004, median follow up from ASCT is 59 months, range 8-107 months. All pts had the same pretransplant therapy and were transplanted to one year after diagnosis. Results. Following ASCT, 52 pts (29%) were in complete remission (CR) and 113 pts (62%) in partial remission (PR). The median progression-free (PFS) and overall (OS) survival from transplant were 26.7 and 72.6 months, respectively. Seventeen pts (9%) are in CR and disease-free over 5 years after ASCT (median follow up of this subgroup is 87 months, range 63-112). Differences in survival among pts with clinical stages according to DS system were not statistically significant (p=0.214). Patients with clinical stages according to ISS were statistically significant (p=0.021). Patients with clinical stages according to ISS were significant differences in survival (p under 0.001): stage I (71 pts) ‘ median was not yet reached, stage II (70 pts) - median 72 months, stage III (25 pts) median 26.0 months. Significant prognostic parameters for poor survival were: age at transplant over 60 years (p under 0.001), IgA typ of monoclonal immunoglobulin (p=0.036), renal impairment with serum creatinine at diagnosis over 2 mg/dL (p=0.007), no achievement of CR after ASCT (p under 0.001). The status of disease before ASCT and type of maintenance therapy after transplant (alone interferon (IFN), IFN + dexamethasone, 4 cycles of chemotherapy CED) and after it IFN) did not significantly affect OS after ASCT. Conclusion. In our group of patients the survival after ASCT correlated with the stage according to ISS, age, clinical response after transplant, type of paraprotein and renal impairment at diagnosis. The most significant parameters for better survival of MM pts after transplant are: age under 60 years at transplant, no ISS stage III at diagnosis and achievement of CR after transplantation.
**Bortezomib in Relapsed Multiple Myeloma: Response Rates and Duration of Response Are Independent of Chromosome 13q**

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**Background.** Presence of a chromosome 13q-deletion confers a poor prognosis to patients (pts) with multiple myeloma (MM), even in the context of intensive treatment programs and thalidomide. Bortezomib is the first compound of a new class of agents the proteasome inhibitors showing activity in relapsed and chemotherapy-refractory MM. Results of SUMMIT and APEX trials suggested that bortezomib is active in MM with previously recognized unfavorable prognostic factors. **Aims.** To study the activity of bortezomib in relapsed MM and potential associations with prognostic factors (standard clinical parameters and chromosomal aberrations: deletion of chromosome 13q14 [del(13q14), 14q- translocations [t(14q23), gain of 1q21]). **Patients and Methods.** We evaluated 51 consecutive pts with relapsed/refractory MM (median number of prior therapies: 3; 92% had high-dose, pulsed dexamethasone, 71% thalidomide, 45% high-dose therapy; median time from first line therapy to bortezomib, 4.8 years). Treatment consisted of single agent bortezomib according to the standard regimen (1.3 mg/m² on days 1, 4, 8, 11; Q21 d). Genomic abnormalities were determined by means of interphase FISH. **Results.** Similar response rates to bortezomib were observed in pts with del(13q14) (13 of 26 pts = 50%) and with normal chromosome 13q (15 of 25 pts = 60%) (p=0.34). Of note, rates of CR/nearCR were also not different between the two patient populations (25% vs. 16%). Moreover, median duration of response was 10.4 months in pts with del(13q14) compared with 9.3 months in pts with normal 13q-status (p=0.29). Only those pts with del(13q14) who did not show a response to bortezomib experienced a rapidly progressive clinical course leading to overall shortened survival. For an improved identification of such pts, additional parameters were tested. In a subset of pts, analyses for gain of 1q21 (C-KS1B gene) were performed. Among 10 pts with simultaneous del(13q14) and gain of 1q21, 8 failed to respond to bortezomib, and their median survival was only 3.3 months. We also observed that pts with low serum levels of albumin had a poor outcome after bortezomib. Thus, pts not benefiting from single-agent bortezomib were characterized by the combined presence of a del(13q14) and low serum albumin levels (median survival 5.3 months). A 3% drop of microglobulin, however, was not important for treatment outcome after bortezomib. Finally, 3 pts were found to have a t(4;14)(p16;q23) in addition to a del(13q14), and all of them had a > 50% reduction of their paraprotein after bortezomib. **Conclusion.** Our results indicate that bortezomib has good clinical activity in MM patients with high-risk cytogenetic features. The simultaneous occurrence of a del(13q14) with gain of 1q21 and/or low serum albumin allows for the identification of pts not benefiting from single-agent bortezomib. It is suggested to evaluate bortezomib combinations in such pts.

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**Efficacy and Safety of Melphalan/Arsenic Trioxide/Ascorbic Acid Combination Therapy (MAC) in Patients with Relapsed/Refractory Multiple Myeloma: A Prospective, Multicenter, Phase II, Single-Arm Study**

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**Background.** Multiple myeloma (MM) is an incurable B-cell malignancy, and nearly all patients develop renal insufficiency during the course of their disease. Among patients who develop renal insufficiency, which is associated with a poor survival. Thus, it is imperative to explore new therapeutic options that can improve their renal function and prognosis. Arsenic trioxide (ATO) is an active anti-MM agent. In preclinical MM studies, the addition of ATO to the cytotoxic agent melphalan overcomes resistance to this alkylating agent. Moreover, ascorbic acid (AA) enhances the cytotoxic effects of ATO. Importantly, a small pilot clinical study of melphalan, ATO, and AA (MAC) therapy for relapsed and refractory MM patients has shown that this combination is well tolerated, with significant durable responses as well as improved renal function for the patients with baseline azotemia. **Aims.** The primary objectives were to determine response rate, time to progression, and safety and tolerability of MAC therapy in a larger multicenter trial. The secondary objectives were to analyze time to response, progression-free survival, overall survival, and the effects of MAC therapy on renal function. **Methods.** Patients with relapsed or refractory MM...
received melphalan (0.1 mg/kg PO), ATO (0.25 mg/kg IV), and AA (1 g IV) on days 1-4 of week 1, AT0 and AA twice weekly on weeks 2-5, and rest during week 6 of cycle 1; melphalan on days 1-4 and AT0 and AA twice weekly on weeks 1-5, and rest during week 6 of cycles 2-6. Results. Patients (N = 65) had a median of 4 (range, 1-8) prior therapies, including melphalan, bortezomib, thalidomide/lenalidomide, glucocorticosteroids, and autologous stem cell transplantation. Objective response rates were observed in 31 (48%) patients, including 2 complete (CR), 15 partial (PR), and 14 minor responses (MR). The median time to progression, time to response, and overall survival were 7 months (0.25-24 months), 2 months (2-6 months), and 19 months (2-27 months), respectively. Notably, of the 25 patients who had elevated baseline serum creatinine (SCr) levels, 17 (68%) showed improvement in renal function. Grade 3 or 4 anemia and/or neutropenia occurred in 3 patients and 1 patient, respectively. Common grade 3 or 4 nonhematologic adverse events were fever/chills (15%), pain (8%), and fatigue (6%). Two patients had single occurrences of prolonged QTc interval (498 and 502 msec) resulting in a brief delay in ATO administration, but continued ATO dosing was not accompanied by any further episodes of QTc prolongation. One patient developed unstable bradycardia without a prolonged QTc following the first ATO infusion and was removed from the study. Conclusions. The MAC combination regimen was an effective treatment for patients with relapsed or refractory MM, producing objective response rates of the patients in this heavily pretreated group. Patients with renal insufficiency at baseline showed improvements in renal function with MAC therapy. The MAC regimen was well tolerated, with relatively few grade 3 or 4 hematologic adverse events or cardiac events. These results show that the MAC combination regimen is an effective and well-tolerated new therapeutic option for patients with relapsed or refractory MM.

0231

INSULIN-LIKE GROWTH FACTOR 1 (IGF-1) IS OVEREXPRESSED IN MULTIPLE MYELOMA PLASMA CELLS (PC) AND REGULATES THE EXPRESSION OF THE IGF-1 RECEPTOR

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Background. IGF-1 plays an important role in regulating cell proliferation, differentiation, apoptosis, and transformation. Recent studies have shown that IGF-1 is an important survival and growth factor in multiple myeloma (MM). Moreover, IGF-1 down-regulates IGF-1R expression at the transcriptional level, by autocrine or paracrine mechanism. Method. IGF-1 and IGF-1R expression by autocrine or paracrine mechanism was evaluated by quantitative real-time PCR in a series of 58 newly diagnosed MM patients primarily treated with thalidomide and dexamethasone. For each patient, we isolated the CD138+ cell fraction from bone marrow (BM) sample at diagnosis and, in 24/53 patients for whom material was available, also at the end of induction therapy. CD138+ CD19+ and CD138+ CD19- subsets were evaluated. A pool of donors was used as calibrator. The Mann-Whitney and the Spearman Rank Correlation tests were applied for statistical analysis. Aim. Correlate the expression of these genes with presenting karyotypic features of MM patients and evaluate their relationship with response to therapy. Results. Both neoplastic PC and CD138- cell fractions expressed a markedly high levels of IGF-1 (median 145.01 and 3.07, range 0.04-10.78 and 0.21-13.55, respectively). Expression of IGF-1R was significantly increased in CD138+ cells (1/IGF-1R pathway may exist between different genetic subtypes. The ability to efficiently regulate IGF-1R expression may thus have an important prognostic value. Moreover, a different regulation of IGF-1/IGF-1R pathway may exist between different genetic subtypes. The study of post transduction modifications of the IGF-1R will be needed, in order to get more insight into the relationship between the IGF-1 and IGF-1R expressions and IGF-1R activation.

0232

AUTOIMMUNITY IS ASSOCIATED WITH BETTER SURVIVAL IN MULTIPLE MYELOMA

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Background. In Western countries, multiple myeloma (MM) is the second most common hematopoietic malignancy after non-Hodgkin lymphoma. Autoimmunity remains yet unexplained in the progression of B-cell malignancy. The median survival of 3 to 4 years. Autoimmunity is associated with improved outcome in patients with certain tumors suggesting that host-related immune response plays an important role in the pathogenesis. Given the association between MM and certain autoimmune diseases, documenting the impact of autoimmunity on MM survival might provide clues to identifying the host-related immune response to autoimmunity. Aim. To assess the prognostic significance of autoimmunity in patients with MM. Methods. Records on 10,557 population-based MM patients reported to the Swedish (1964-1990) and Danish (1977-1997) Cancer Registries were linked to the nationwide inpatient Registries to capture hospital records including data on 32 defined autoimmune disease and the Cause of Death Registries to retrieve mortality data. Using logistic regression models adjusted for gender, age, and country, we defined risk of MM mortality in relation to presence/absence of any autoimmune disease and for categories of autoimmune conditions: (Group A) autoantibodies (AAB) with systematic involvement, (Group B) AAB with organ-specific involvement, and (Groups C) no AAB. We also evaluated risk of MM mortality for individual autoimmune conditions. Based on the expectation that secular trends in MM and autoimmune disease diagnostics/treatment could introduce heterogeneity we explored models stratified by calendar-period and calendar-strata. Results are presented by the approximate mid-point of year of MM diagnosis (<1987 vs. ≥1987) strata. Results. In the first calendar-period (<1987) we observed overall significantly decreased risk of MM mortality among persons with presence (n=575) of any autoimmune disease (OR=0.44, 95% CI 0.25-0.79). When we fit models by the 3 autoimmune disease categories, we observed decreased MM mortality for each: Group A (OR=0.34, 95% CI 0.15-0.75), Group B (OR=0.71, 95% CI 0.25-1.97), and Group C (OR=0.32, 95% CI 0.18-0.82), although only Groups A and C reached formal significance. The Group A effect was driven by the conditions rheumatoid arthritis and polymyositis/dermatomyositis; and the Group C effect was driven by conditions including ankylosing spondylitis, rheumatic fever, sarcoidosis, and polymyalgia rheumatica. In the second calendar-period (>1987), we found no statistical associations between MM mortality and presence of any autoimmune disease (OR=1.20, 95% CI 0.92-1.56), Group A (OR=1.25, 95% CI 0.78-2.01), Group B (OR=1.39, 95% CI 0.87-2.21), Group C (OR=0.97, 95% CI 0.66-1.45), or individual autoimmune conditions. Estimates were similar when analyses were restricted to autoimmune diseases documented only prior to MM diagnosis (n=520). Summary/Conclusions. The decreased mortality among MM patients diagnosed in the first calendar-period with certain autoimmune diseases is intriguing and provides support for the role of host-related immunity as an anti-tumor agent in MM therapy. The observed protective effect in the first (1964-1986), but not in the second (1987-1998), calendar-period might reflect variations in diagnostic procedures, clinical management, and or treatment strategies for autoimmune disease and/or MM. Future studies are needed to clarify underlying mechanisms of our findings.
BORTEZOMIB AND HIGH DOSE MELPHALAN: A NEW CONDITIONING REGIMEN BEFORE AUTOLOGOUS STEM CELL TRANSPLANTATION FOR MULTIPLE MYELOMA


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Background. The achievement of Complete Response (CR= disappearance of M component by immunofixation) or Very Good Partial Response (VGPR= >90% of M component reduction) is the main prognostic factor for survival after Autologous Stem Cell Transplantation (ASCT) in Multiple Myeloma (MM). High Dose Melphalan (HDM) (200mg/m²) is the recommended conditioning regimen before ASCT. However, the rate of CR+VGPR is >40% to 50%. Bortezomib (BOR) has demonstrated a significant activity in relapsed/refractory patients, a synergistic effect with Melphalan (MEL) and a lack of haematological toxicity. Aims. The combination of BOR and HDM was a logical approach to improve the rate of CR+VGPR after ASCT. Methods. Between June 2004 and February 2005, 25 patients with stage II or III DS MM have been enrolled to receive an ASCT conditioned with both BOR and HDM. BOR (1mg/m²/d) was delivered on days -6, -3, +1 and +4, MEL (200mg/m²) was administered on day -2, and blood stem cells (median 3.5 x10^6 CD34/kg) were infused on day 0. The dose of BOR was initially started to 1.3 mg/m²/d for 3 patients but was lowered because of neuropathy. Results. The main characteristics of the 25 patients were: median age = 56 years; M component= IgG in 17 cases (68%); M component= IgA in 4 cases; chromosome 13 deletion in 6 cases on 13 assessable. Seven patients were in MR or with a progressive disease (PD) after a first response (PR= > 50% of M component reduction). CR and 6 (46%) a VGPR. Two patients were non responders. Among the 4 patients receiving a second ASCT, 2 CR and 1 VGPR were observed after BOR+HDM at 3 months. Conclusions. The preliminary results strongly suggest that BOR (1 mg/m² x4) and HDM is a safe and highly effective conditioning regimen in MM, requiring further investigations.

INTERMEDIATE-DOSE MELPHALAN (100MG/M²), THALIDOMIDE, DEXAMETHASONE AND STEM CELL SUPPORT IN PATIENTS WITH REFRACTORY OR RELAPSED MYELOMA

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Background. Combination approaches of new drugs with conventional therapies have increasingly been adopted as savage or even first-line treatment for multiple myeloma. High-dose or dose-intensive i.v. melphalan followed by hematopoietic cell support induced higher response rates and improved outcome compared to conventional oral melphalan in several randomized trials. These findings formed the rational for the combination of both bortezomib and thalidomide with intermediate-dose melphalan (100mg/m²) as conditioning regimen prior to autologous hematopoietic cell infusion. No data are available on the use of this combination as conditioning regimen in the transplant setting. Aims. We assessed the safety, tolerability and response rate of intermediate-dose melphalan, Velcade, thalidomide and dexamethasone followed by stem cell support in refractory or relapsed multiple myeloma (MM) patients. Methods. Twenty-six advanced myeloma patients were treated with melphalan at 50 mg/m² and bortezomib at 1.5 mg/m² on days -6 and -3 associated with thalidomide at 200 mg and dexamethasone at 20 mg on days 6 through -3 (MVDt), followed by hematopoietic cell support on day 0. Results. Between September 2004 and December 2005, 26 patients with relapsed or refractory MM were enrolled in the study. Median time from diagnosis was 48,5 months (range 2,3-142 months). All patients were induced with standard autologous transplants. Moreover, 14 (54%) were treated with a combination of thalidomide and dexamethasone and 13 (50%) with a second autologous transplant as salvage treatments. Objective responses occurred in 17 of 26 patients (65%), including one complete remission (CR 3%), 3 near complete remissions (nCR, 11%) and 2 very good partial response (VGPR 7%); 3 patients (10%) showed minimal response. Six patients (23%) showed no response (NR) and no patients showed progressive disease (PD). Interestingly, of 5 patients who had previously progressed while on thalidomide and prednisone, 1 reached nCR, 2 PR and 1 MR. After a median of 9 months (range 1-16), 7 patients (27%) were alive in remission, 15 patients (58%) relapsed, 4 patients (15%) died from progression disease and one patient from infective toxicity. Median progression-free survival for all patients was 6 months (range, 1 to 16 months). Response rate was higher than that induced by the previous line of treatment in 12 patients (46%); response duration was longer than in 6 patients (30%). Grade 3 thrombocytopenia developed in 46% of patients, grade 4 in 54%. Forty-two percent of patients showed grade 3 or 4 neutropenia. No treatment-related grade 5 adverse events were observed. No serious bleeding was observed during both aspirin and enoxaparin prophylaxis. Conclusion. MPT with enoxaparin and RMP with aspirin were safe and equally effective in reducing the risk of recurrent VTE to levels observed in patients who received oral MP only.

ENOXAPARIN OR ASPIRIN FOR THE PREVENTION OF RECURRENT THROMBOEMBOLISM IN NEWLY DIAGNOSED MYELOMA PATIENTS TREATED WITH MELPHALAN AND PREDNISONE PLUS THALIDOMIDE OR LENALIDOMIDE

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Background. Venous thromboembolism (VTE) is a common complication in cancer patients. The risk is particularly high after surgery, during chemotherapy and in association with central vein catheters. Aggressive antitumor therapy with thalidomide or lenalidomide increases the risk of thrombosis. The underlying mechanisms are poorly understood, but these therapeutic agents induce vascular damage. The risk of thrombosis is higher for patients receiving thalidomide at diagnosis in comparison with those treated at relapse. Low-molecular weight heparin is considered the standard prophylaxis in these patients. Low-intensity warfarin and aspirin have also been used. Patients received melphalan, prednisone (MP) alone; or MP plus thalidomide (MTP); or MP plus lenalidomide (Revlimid®) (RMP) and aspirin. Aims. We evaluated the efficacy and safety of enoxaparin or aspirin in the prevention of VTE, in newly diagnosed myeloma patients. Methods. In the MP group, no patient received anticoagulant prophylaxis. In the MTP group, MP was combined with Thalidomide (Pharmion Ltd., Cambridge, UK), no anticoagulant prophylaxis was administered until December 2003. In a preliminary analysis, an high incidence of thrombosis was observed, therefore the protocol was amended and enoxaparin at 40 mg per day was introduced as prophylaxis and delivered subcutaneously during the first four cycles of MP regimen. In the MP group, all patients received aspirin 100 mg once a day continuously until any sign of relapse or progressive disease. The time to occurrence of the first thromboembolism was calculated from the start of chemotherapy. Results. In the MP group, VTE was reported in 2 of the 144 patients; in the MTP group, symptomatic deep-vein thrombosis, pulmonary embolism, or both occurred in 12 of the 65 patients who did not receive any anticoagulant prophylaxis. Thromboembolism was observed in 4 of the 78 MP patients who received enoxaparin prophylaxis; in RMP group, one of 50 patients, who received aspirin, experienced pulmonary embolism. Median time for VTE was 4 months in the MP group, 3 months for MTP with and without anticoagulant prophylaxis. In the RMP group, the only episode of thromboembolism occurred after 1 months from start of therapy. In comparison with MP, the hazard ratio for recurrent VTE in the MTP group without any prophylaxis was 14.5 (95% CI, 5.2 - 64.3; p<0.0001); in the MTP group with enoxaparin it was 3.76 (95% CI, 0.69 - 20.52; p=0.11); in the RMP group, with aspirin it was 0.15 - 19.3; p=0.67). No significant interactions between treatment group and risk factors were detected. No serious bleeding was observed during both aspirin and enoxaparin prophylaxis. Conclusion. MPT with enoxaparin and RMP with aspirin were safe and equally effective in reducing the risk of recurrent VTE to levels observed in patients who received oral MP only.
patients developed grade 3 anemia, 38% grade 4, whereas all patients showed grade 4 neutropenia. Five patients (19%) showed grade 1-2 neurologic toxicity, 1 patient grade 3. Infections consisted of pneumonia in 9 patients (35%), fatal for one patient and neutropenic fever (12%). Infections required iv broad spectrum antibiotic therapy in 50% of the patients. Conclusion. MVTD showed encouraging activity with manageable toxicity and represents a promising treatment for advanced myeloma patients.

**0236**

**THALIDOMIDE-DEXAMETHASONE VS THALIDOMIDE-DEXAMETHASONE AND PEGYLATED LIPOSOMAL DOXORUBICIN: A CASE-MATCHED STUDY IN PATIENTS WITH ADVANCED MULTIPLE MYELOMA**

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Background. Thalidomide alone or in combination with dexamethasone and/or chemotherapy is the most extensively used compound in the treatment of relapsed/refractory multiple myeloma (MM). However, this thalidomide-based regimen is more effective and less toxic is still unknown. Recently we demonstrated that the combination of thalidomide, dexamethasone and pegylated liposomal doxorubicin (ThaDD) leads to high rate and high quality of response (ORR=92%; CR/nCR=32%) with a PFS of 47% and OS of 65% at 2 years. Aims. In the present study we compared ThaDD with the combination thalido mide-dexamethasone (T-D), frequently used in advanced MM patients as salvage therapy. Methods. A total of 47 relapsed/refractory patients treated ThaDD was compared with a control group of 47 pair matched patients for age, serum β2-microglobulin, previous chemotherapy and high-dose therapy. ThaDD regimen consisted of thalidomide 100 mg/day continuously, dexamethasone 40 mg on days 1-4 and 9-12, pegylated liposomal doxorubicin 40 mg on day 1 every 28 days. T-D regimen consisted of thalidomide 100 mg/day continuously and dexamethasone 40 mg on days 1-4 repeated monthly. Both groups included a lot of elderly patients, who had received 3 or more prior chemotherapy regimens and who had undergone stem cell transplantation. Results. ThaDD significantly increased overall response rate in comparison with T-D (92% vs 63.5%; P=0.008) and, importantly, induced significantly better quality of response (≥PR 75.5% vs 59.5%, P=0.077; ≥VGPR 36% vs 15%, P=0.018; CR/nCR 30% vs 10.5%; P=0.021). Compliance to therapy was satisfactory in both groups of patients and grade 3-4 neurological toxicity were limited (4.2% in patients treated with ThaDD vs 2.1% in those receiving T-D). On the contrary, grade 3-4 hematological toxicity (32% vs 0%; P<0.0001), grade 3-4 infections (23% vs 0%; P<0.0001) and vascular events (12.8% vs 6.4%; P=0.293) were more frequent in patients treated with ThaDD although no deaths were related to these complications. The rate of infections decreased below 10% when ciprofloxacin was added in the ThaDD regimen. The median PFS was significantly longer in ThaDD group (22 months vs 11.5 months, 36% vs 13% at 3 years; P=0.0008) as well as median EFS (21 months vs 11.5 months, 28% vs 13% at 3 years; P=0.0077) and OS (NR vs 23.5 months, 52% vs 26% at 3 years; P=0.051). Conclusions. ThaDD, as salvage therapy for MM, regimen is superior to the combination thalidomide-dexamethasone since it induces a significantly higher and better quality response rate than T-D and this translates into a significantly better survival measures. The incidence of infections and deep venous thrombosis are more frequent in ThaDD group but they result manageable with adequate prophylaxis. We believe that ThaDD combination could be a valid candidate for comparison with bortezomib- or lenalidomide-based regimens in order to identify the optimal salvage therapy in advanced MM.

**0237**

**DYSFUNCTION OF TOLL-LIKE RECEPTORS: A POSSIBLE IMPLICATION IN THE PATHOGENESIS OF IMMUNODEFICIENCY IN MULTIPLE MYELOMA**

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Background. Immune paresis, renal failure, neutropenia and anti-myeloma therapy can combine to cause severe immunodeficiency in multiple myeloma (MM). Thus, infections are a major cause of death in MM. There is limited information for the possible role of innate immunity in the pathogenesis of immunodeficiency in MM. Innate immunity provides a first line of host defence against infection through microbial recognition and killing while simultaneously activating a definitive adaptive immune response. Innate immune detection of pathogens relies on specific classes of microbial sensors, such as Toll-like receptors (TLRs). TLRs are principal mediators of rapid microbial recognition and function mainly by detection of structural patterns that do not exist in the host. Aims. The aim of this study was to evaluate the expression and function of TLRs in newly diagnosed MM. According to our knowledge, such information is not available in the literature. Patients and Methods. Twenty-two patients with MM at diagnosis (15M/9F; median age 68 years), 2 patients with MGUS and 11 healthy, age- and gender-matched controls were studied. Five patients had stage 1, 9 stage 2 and 8 stage 3 myeloma, according to ISS. After the collection of peripheral blood, mononuclear cells (PBMCs) were isolated by ficoll centrifugation (Histopaque-1077, Sigma-Aldrich). These cells were measured for the expression of TLRs (antibodies from ebioscience) using fluorescence activated flow cytometry (FC 500, Beckman Coulter). In addition, 1×10⁶ cells/mL were cultured in 5% FCS 1% pen/strep RPMI in the presence or absence of various TLR ligands and supernatants collected after 20h. These were examined for the presence of inflammatory cytokines (tumor necrosis-α, TNF-α, and interleukin-6, IL-6) by ELISA (Becton Dickinson). Results. We found that although patients with MM express TLRs in PBMCs, their response to certain TLR ligands is defective when compared to healthy controls. TLR2, TLR4 and TLR6 of PBMCs of healthy controls reacted normally to their ligand R-848 (Imiquimod) to secrete high levels of TNF-α (medi-an value for patients and controls: 4.3 and 4.5 ng/mL, respectively; p=NS). NOD1, another pattern recognition receptor that recognizes bacterial peptidoglycans also reacted normally in MM patients. Similar observations have been made for the expression of IL-6. Our preliminary analysis showed that there was no difference in terms of TLRs function between MGUS patients and controls and between myeloma patients of different disease stages. Conclusions. There is a significant defect in TLR function in patients with MM, especially of these involved in immunity against bacterial infections. Thus the immune system fails to receive early priming signal which may contribute to the increased infections observed in MM. The restoration of function of TLRs to their normal levels has the potential to improve bacterial immunity in MM patients.
Myeloma and other monoclonal gammopathies II

FLUORODEOXYGLUCOSE POSITRON EMISSION TOMOGRAPHY IN MULTIPLE MYELOMA, SOLITARY PLASMACYTOMA AND MONOCLONAL GAMMOPATHY OF UNKNOWN SIGNIFICANCE

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The aim of our study was to evaluate the role of fluorine-18 fluorodeoxyglucose positron emission tomography (FDG-PET) in plasma cell malignancies. A total of 49 patients were enrolled including 13 patients with newly diagnosed multiple myeloma (MM) and negative bone radiographs, four patients with solitary plasmacytoma, 26 patients with MM in remission but with suspected relapse, and six patients with monoclonal gammopathy of unknown significance (MGUS) with suspected progression to MM or with suspected other malignancy. FDG-PET results were verified by conventional imaging methods, including plain radiographs, magnetic resonance imaging (MRI) and computer tomography (CT). Focally increased FDG uptake was observed in three (23%) of 11 newly diagnosed myeloma patients with negative bone radiographs. The findings were all confirmed by CT or MRI. FDG-PET was negative in two patients with newly diagnosed MM, negative bone radiographs, and without focal infiltration on MRI but with anemia, high monoclonal immunoglobulin and high bone marrow infiltration by plasmocytes. In all other cases FDG-PET negativity in asymptomatic patients was associated with favorable prognosis; these patients are without progression after the median follow-up of 14 months. Focally increased tracer uptake was found in five of 26 patients with MM in remission. In four cases it was due to MM relapse, in one case due to ovarian carcinoma. Only in one patient FDG-PET failed to recognize extramedullary progression. Of the 20 patients who had negative FDG-PET scans, only one relapsed 12 months after FDG-PET examination; the remaining 19 patients are without progression with the median follow-up of 15 months. FDG-PET was positive in two of six patients with MGUS. In one case a thyroid carcinoma was later detected, in the other an intestinal tumor was found. We conclude that FDG PET might contribute to initial staging of MM patients with negative bone radiographs and is useful for the follow-up of patients in remission especially in non-secretory MM and in patients with large plasmocytoma (>5 cm) after radiochemotherapy.
effects were constipation (10 patients WHO grade 1, 8 patients WHO grade 2), polyneuropathy (14 patients WHO grade 1, 2 patients WHO grade 2) and somnolence (4 patients WHO grade 1). None of the 23 patients developed dose-limiting hematotoxicity as defined by an ANC < 1.0 Gpt/L for > 7 days or an ANC < 0.5 Gpt/L for > 3 days or platelet count < 25 Gpt/L. Short neutropenia was reported in 8 patients (WHO grade 3 and 4) but no thrombocytopenia was observed. BPT with a dose between 50 and 200 mg thalidomide daily is well tolerated in patients with relapsed or refractory MM.

0241.
CANTHARIDIN, A DERIVATIVE OF BLISTER BEETLES INDUCES APOPTOSIS IN MULTIPLE MYELOMA CELLS VIA INHIBITION OF IL-6-INDUCIBLE STAT3 PATHWAY: NEW AGENT FOR SIGNAL TRANSDUCTION THERAPY OF MULTIPLE MYELOMA
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Background. Multiple myeloma remains incurable despite the use of high-dose chemotherapy with hematopoietic stem cell transplantation; therefore, novel therapeutic approaches are urgently needed in clinical settings. The understanding that has recently been gained into the biology of myeloma has led to the development of biological treatments, which target the myeloma cells and its microenvironment. These agents have shown remarkable activity against refractory myeloma in early clinical trials, but prolonged drug exposure may result in the development of drug resistance and improve the clinical outcome of patients. Aims. The purpose of this study was to evaluate whether CTD inhibited IL-6-induced apoptosis in myeloma cells and to determine its mechanism of action.
Methods. To address our hypothesis, we conducted a series of experiments using the IL-6-mediated STAT3 signaling pathway. The results of these experiments are presented in this paper.
Results. CTD inhibited STAT3 phosphorylation and induced apoptosis in myeloma cells, and these effects were dose-dependent. The effects of CTD on cell growth, apoptosis, cell cycle status, and the signaling pathway were studied. CTD inhibited cellular growth of myeloma cells as well as freshly isolated myeloma cells from patients with MM. The effects of CTD on cell growth, apoptosis, cell cycle status, and the signaling pathway were studied. CTD inhibited cell cycle arrest, but induced apoptosis of myeloma cells and primary myeloma cells from patients with MM. In conclusion, we report here for the first time that CTD inhibits IL-6-induced apoptosis in myeloma cells, and that it may be a new therapeutic agent for signal transduction therapy of myeloma.

0242.
CITRULINE CONCENTRATION AFTER HIGH-DOSE MELPHALAN IN AUTOLOGOUS HSCT RECIPIENTS
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Background. Mucosal damage to the intestines induced by intensive myeloablative conditioning for an allogeneic HSCT can be determined by the concentration of citrulline which is a functional marker of small intestinal enterocytes. However, there are no data available about the kinetics of citrulline levels following high-dose melphalan used to prepare for an autologous HSCT. Aims. We were interested to know whether and when the citrulline concentrations declined after starting myeloablative therapy. Methods. We selected 29 patients who underwent an autologous HSCT following conditioning with HDM 100 mg/m² HSCT day -3 & -2. We collected plasma samples from each patient via a central venous catheter at 9:00 hour on the first day of conditioning therapy and 3 times per week (Monday, Wednesday, Friday) thereafter until discharge. The samples were stored frozen until citrulline concentrations could be determined by HPLC. A1 oral mucositis was registered using a Daily Mucositis Score. Results. The baseline mean citrulline concentration was 28 mM which is lower than the 35 mM that is found normally. The mean citrulline concentrations declined rapidly thereafter reaching a nadir of 6.7 µmol/L 11 days after starting HDM which is HSCT day +7. Citrulline concentrations then only increased gradually and were still significantly low at 12 mM when patients were discharged. The most severe oral mucositis coincided with the nadir of citrulline. Conclusion. Citrulline appears to be an excellent marker of small intestinal mucosal barrier function induced by HDM to prepare for an autologous HSCT.

0243.
PLASMA CELL PROPIDIUM IOIDE (PC-PI/CD138) AND ANNEXIN V (PC-AI/CD138) INDICES IN MULTIPLE MYELOMA AND MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE EARLY PREDICTORS OF TRANSFORMATION?
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Background. Propidium iodide and annexin V indices have a close relation to progression in multiple myeloma (MM) and have been proved to be significant and independent prognostic factors in its evaluation. The aim of this study is to evaluate these indices also in monoclonal gammapathy of undetermined significance (MGUS) in correlation with MM, especially with stage I (D-S) to determine their importance as early predictors of transformation from MM into symptomatic phase of the disease. Methods. Analysed group consists of 257 patients (70 MGUS and 187 MM patients -21 st. I, 78 st. II and 88 st. III), all at the time of diagnosis, before therapy. 20 of MGUS patients were measured also in regular 6-12 month intervals to evaluate the course of MGUS. Proliferative activity of plasma cells was measured using propidium iodide index (PCPI/CD138), rate of apoptosis using annexin V index (PC-AI/CD138), followed by method of flow-cytometry (DNA-Prep Reagents Kit, Coulter, Software Multicycle Fry. Phoenix). For statistical estimation Student’s-t-test, ANOVA and non-parametric Mann-Whitney test were used. Results. Within the evaluation of propidium iodide and annexin V in MGUS and MM there was a significant difference between the values of both indices in MGUS the values of proliferation were lower (M: 1.9% vs MM: 2.6%, p<0,0001) and the values of apoptosis were higher (M: 7.45% vs MM: 9.5%, p<0,0001). If we depicted only stage I MM there was also statistically significant difference in proliferation when M: 2.5% vs MM: 3.0% (p<0,016). In apop- tosis we found higher values in MGUS (M: 7.45% vs MM: 6.2%), however not statistically significant (p=0,121). In next step we tried to analyse differences in PC-PI and PC-AI within the stages of MM. The corresponding medians of PC-PI were for stage I, II and III (D-S) values 2.5%, 2.6% and 2.7%, none of them being significant (p=0,589). For apoptosis the
results were similar - for stage I, II and III values 6.2%, 4.9% and 4.3%, p<0.06. Finally, we compared the values of PC-I and PC-AI within the course of 20 MGUS patients - there was no statistical significance between the values, either. Conclusion. Our measurements support the hypothesis of PI-C and PC-AI being independent prognostic factors and also the indicator of early transformation of MGUS into MM. Within the course of MGUS there exists no significant change in either of the indices. In transforming MM patients, however, the rate of PC-AI in MM, there is a significant increase in PC-AI together with decrease of PC-AI. The above results also confirm the major importance of proliferation in the process of transformation into MM - there is significant difference even between MGUS and stage I MM, on the other hand, decrease in apoptosis predominates for the first 6 months of this process. Measurement of proliferation and apoptosis contributes to the assessment of MM prognosis, and plays also a prominent role in the evaluation of the course of MGUS, especially as an early predictor of transformation into multiple myeloma.

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0244

EPIDEMIOLOGY OF ANEMIA IN 720 PATIENTS WITH MULTIPLE MYELOMA: RESULTS FROM EUROPEAN ANAEMIA SURVEY

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Background. Although anemia is a common complication of multiple myeloma (MM) patients (pts), information on the evolution of anemia during follow up, relation with age and performance status, risk factors for anemia and treatment practices was not available. Aims. Identify the incidence, prevalence and evolution of anemia during an up to 6 months follow up period, analyse possible correlations between anemia and clinical characteristics, identify risk factors for evolution of anemia (in pts with myeloma and lymphoma) and study patterns of anemia treatment in European myeloma pts. Methods. 720 patients with multiple myeloma (male 52% and female 48%) were enrolled into a prospective, epidemiologic survey, ECAS (European Cancer Anemia Survey), which included an additional 1640 pts with lymphoma (L) and a total of 15,370 pts with cancer at any stage of their disease. Survey data were collected for up to 6 data points or 6 months of scheduled visits (Ludwig, EJC 2004; 40 (15): 2299-2307). Results. Median age in MM pts was 65.7 years (range 31-94), with 26% of patients presenting with age <60 years (yrs), 52% with age 60-69 yrs, and 40% with age <60 yrs. 28% of the 720 pts with MM were newly diagnosed, 53% persistent/recurrent disease and 17% were in remission. In terms of cancer treatment, 50% were receiving chemotherapy (CT), 46% were not receiving any cancer treatment, with the remainder receiving radiotherapy or concomitant CT and radiotherapy. At enrollment, 69% of patients were anemic (Hb <12g/dL), 30% had Hb < 10 g/dL and 39% Hb of 10 to 12 g/dL. 85% were anemic at some time during the survey. 78% of those <60 yrs, 85% of those 60-69 yrs and 90% of those 70+ were ever anemic. 44% had a WHO score of 2-4. The incidence of anemia in MM who were not anemic at enrolment and who started CT during ECAS was 75%. Incidence of anemia increased with increasing age (60% in pts < 60 yrs, 88% in those 60-69 yrs and 100% in those 70+). Adverse WHO score correlated with low Hb (r=0.346). Despite the 59% of those who became anemic having a nadir Hb < 10 g/dL, 41% received no anemia treatment, 2% received iron, 22% transfusion and 35% received epoetin. Logistic regression analysis of MM/L pts revealed 4 variables significantly predicting anemia development: Initial Hb (adjusted odds ratio (AOR) 4.2), persistent/recurrent disease (AOR 1.5), female gender (AOR 2.8), and treatment with platinum-based CT (AOR 5.5) were found to independently predict anemia (p<0.001). Conclusions. Frequency of anemia in MM pts remains substantial and important: prevalence of anemia (ever anemic) was high (85%) in MM pts, increased with age and correlated with poor PS. Follow up during the 6 month post-enrollment period indicated that 75% of initially non-anemic pts developed anemia after starting CT. Anemia treatment was given to 41% of ever anemic MM pts, although 59% had at least once Hb levels <10g/dL. With the identification of important risk factors, anemia management in MM pts could be improved.

0245

A PHASE I/II STUDY OF ARSENIC TRIOXIDE, BORTEZOMIB, AND ASCORBIC ACID IN RELAPSED OR REFRACTORY MULTIPLE MYELOMA

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Background. Arsenic trioxide (ATO), a trivalent arsenite salt, is believed to exert its cytotoxic effect by causing DNA fragmentation characteristic of apoptosis. Clinical studies have shown that ATO has antitumor activity both as a single agent in patients with relapsed or refractory multiple myeloma (MM). Bortezomib (B) is a proteasome inhibitor that is currently approved for the treatment of relapsed or refractory MM. Preclinical studies have shown that combining ATO and B results in synergistic antitumor activity against human MM cells in tissue culture and xenograft animal models. Furthermore, the addition of ascorbic acid (AA) can sensitize human MM cells to the cytotoxic effects of ATO. These observations suggest that the combination of ATO/B/AA may be an effective treatment regimen for patients with MM. Aims. The primary aim of this study was to determine the safety and tolerability of the ATO/B/AA regimen in patients with relapsed or refractory MM. The secondary aims were to determine overall response rate, time to response, time to progression, progression-free survival, and overall survival in these patients. Methods. Patients with relapsed or refractory MM were enrolled in this Phase I/II dose-escalation trial in 6 cohorts. Patients were given ATO (0.125 or 0.250 mg/kg), B (0.7, 1.0, or 1.3 mg/ml), and a fixed dose of AA (1000 mg IV on days 1, 4, 8, and 11 of a 21-day cycle for a maximum of 8 cycles. Results. At the time of this interim analysis, 22 patients (median age, 63 years) have been enrolled, and accrual has been completed on all cohorts. This group had failed a median of 4 (range, 3-9) prior therapies. One occurrence of grade 4 thrombocytopenia was observed. One occurrence of asymptomatic arrhythmia led to patient withdrawal. All other adverse events were grade 1 or 2. For the 21 patients evaluable for efficacy, objective responses were observed in 9 patients (45%), including 2 complete (CR, 10%), 2 partial (PR, 10%), and 5 minor (MR, 24%) responses. Only 1 (1 MR) of 6 patients receiving the lowest dose of B (0.7 mg/ml) showed a response, whereas 4 (1 CR and 3 MR) of 6 patients receiving the middle dose of B (1.0 mg/ml) responded, and 4 (1 CR, 2 PR, and 1 MR) of 9 patients receiving the highest dose of B (1.3 mg/ml) responded. Conclusions. The ATO/B/AA regimen was well tolerated by the majority of patients and produced objective responses in 45% of the patients in this heavily pretreated group. Eight of 15 patients (53%) who previously had had clinical responses to this regimen. The results of this Phase I/II study warrant further clinical evaluation of the ATO/B/AA combination regimen for the treatment of patients with relapsed or refractory MM.

0246

PREVALENCE OF RAS GENE MUTATIONS IN THE CONTEXT OF A MOLECULAR CLASSIFICATION OF MULTIPLE MYELOMA

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Background. Earlier studies have reported that activating mutations involving RA genes, in particular NRAS and KRAS, occur frequently in multiple myeloma (MM). The reported prevalence of mutated tumors varies from 10 to 40% at presentation, rising to 70% at relapse, suggesting a role of this lesion in tumor progression. Notably, the occurrence of such mutation in MGUS and indolent tumors is very low. Mutations of RAS genes, in particular KRAS, but not NRAS, have been found to be associated with higher bone marrow burden and shorter survival. Aims. In the present study we investigated the prevalence and type of RAS mutations in MM in the context of a proposed molecular stratification, named as TC classification, based on the presence of IGH translocation and dysregulation of cyclin D genes in MM. Methods. The presence of NRAS and KRAS gene mutations was investigated in a panel of 82 MM at diagnosis, 13 patients with extramedullary myeloma or plasma cell leukemia, 9 patients with
MGUS and 4 normal controls. The mutation analysis was performed by RT-PCR and direct DNA sequencing on purified CD138+ plasma cell populations (>90%). The expression levels of the three cyclin D genes in MM patients were derived from the gene expression profiling (GEP) data generated using high-density oligonucleotide arrays. GEP data were analyzed using unsupervised (two-dimensional hierarchical clustering) and supervised (SAM, Significant Analysis of Microarrays) approaches. Results. Mutations were found in 16/82 (20%) myeloma patients, in 2/13 (20%) PCL samples and in none of the MGUS patients. In 11 MM patients the mutation involved the NRAS gene at codon 13 (8 patients) and 61 (8 patients), and the KRAS gene at codon 12 (4 patients) and 61 (1 patient), respectively. PCL patients were both harboring a NRAS mutation at codon 61. Mutations were found in patients included in all TC groups: 4 patients in TC1 (28.5%), 5 in TC2 (25%), 3 in TC3 (11.5%) and 2 patients in both TC4 (12.5%) and TC5 (50%) groups. Although the higher frequency of mutations observed in TC1 and TC2 groups, this finding did not reach a significant statistical level. No significant correlation was found with chromosome 13q deletion, trisomy of chromosome 11, or 1q amplification. Unsupervised analysis of gene expression profiles of the 82 patients did not show any particular evidence of clustering of tumors with RAS mutations. A supervised analysis approach, comparing the RAS mutated MM (16) cases versus wild-type (66) tumors in the complete dataset as well as in the TC1, 2, 3 or 4 groups, did not allow the identification of differentially expressed transcripts. Conclusions. Our study confirms the previous evidences reported by us and others and indicates that RAS mutations did not correlate at significant levels with specific genetic lesion or molecular features in MM.

0247 PERSONAL HISTORY OF REPEATED PNEUMONIA IS ASSOCIATED WITH INCREASED RISK OF MULTIPLE MYELOMA

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Background. In Europe and the U.S. a total of more than 35,000 multiple myeloma (MM) cases are diagnosed annually. Although the etiology of MM remains unclear, associations between MM and past history of disorders characterized by chronic inflammation such as pneumonia have been observed in limited clinical and epidemiological studies. Aims. To evaluate risk of MM associated with a personal history of airway infections. Methods. Using population-based linked registry data from Denmark, we conducted a case-control study including 4,476 MM cases diagnosed 1977-1997 and 16,727 age and gender matched controls. All individuals were linked with the Danish Inpatient (1977-1997) and Outpatient (1994-1997) Register to gather information on discharges listing any of the following coded airway infections: tuberculosis, pneumonia, bronchitis, unspecified lower airway infection, laryngitis, nasopharyngitis/pharyngitis, unspecified upper airway infection, sinusitis, otitis media, and influenza. We calculated odds ratios (ORs) and 95% confidence intervals (CIs) as measures of relative risks for each condition using logistic regression. Airway infection data were restricted to those that occurred more than one year before MM diagnosis for cases and their corresponding controls. In models including multiple prior airway infections, we examined the association between MM risk and number of events (1, 2, and 3+) and time from discharge listing a defined airway infection until MM diagnosis (1-5, 5-10, and 10+ years latency). Observed associations were stratified by age at MM diagnosis (<65 vs. ≥65 years with multiple prior pneumonia events). The increased MM risk subsequent to pneumonia was confined to the 1-5 year latency interval suggesting that pneumonia might be a potential late trigger for MM development, rather than a risk-factor for the precursor of MM, monoclonal gammapathy of undetermined significance (MGUS). Alternatively, pneumonia could be a manifestation of immune disturbances in late-stage MGUS. Future studies examining underlying mechanisms of the observed findings may provide insights to the etiology of MM.

0248 MINIMAL RESIDUAL DISEASE CAN BE DETECTED IN ALMOST ALL MULTIPLE MYELOMA PATIENTS IN REMISSION USING A COMBINED APPROACH OF FIVE-COLOUR FLOW CYTOMETRY AND INTERPHASE FISH ON SUBSEQUENTLY SORTED PLASMA CELLS

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Background. Translocations involving IGH, del(13q) and del(17p) are frequently found in multiple myeloma (MM), smoldering myeloma (SM) and monoclonal gammapathy of undetermined significance (MGUS) by fluorescence in situ hybridisation (FISH) and are shown to be of prognostic relevance. However, adequate FISH detection may be hampered by low level bone marrow infiltration, in SM and MGUS and also in MM, at diagnosis and especially during therapy. Plasma cell (PC) targeting strategies are thus mandatory to increase sensitivity of the FISH assay. Aim. We aimed at demonstrating the feasibility and high sensitivity of a combined approach of five-colour flow cytometry (FCM) and interphase FISH on subsequently sorted PC. Therefore, using this approach, we have analysed bone marrow samples from patients at different stages of disease and therapy, all containing low percentages of PC. Methods. A total of 32 bone marrow aspirates were analysed, including samples from patients with MGUS (n=12), SM (n=5), MM at diagnosis or relapse (n=6) and MM in partial or complete remission (CR) (n=11). Cytological analysis of bone marrow smear showed bone PC percentages within the range 0.5% to 11%. Expression of molecules of interest in PC disorders (CD138, CD38, CD56, cytL light chain (λ)) was routinely investigated using five-colour FCM on a FACSAria (BD, US). CD56+GL cell populations were subsequently sorted and spotted on slides with the same instrument. The high purity of the sorted cells (mean 95%) was demonstrated by reanalysing the cells by FCM and by microscopy. Dual colour interphase FISH with probes for IGH, 13q, 17p and 12 (control) (Vysis, Abbott, US) was applied on the sorted PC as well as on the corresponding bone marrow smears. Results. Five-colour FCM showed the presence of an aberrant PC population, by the presence of IgL restriction and/or the expression of CD56 in 27 of 32 samples. On sorted PC, 24 (MM, SMM, MGUS) out of 32 samples (75%), including 4 FCM negative samples, displayed at least one abnormality with FISH (del13q), IGH translocation and del(17p) in respectively 15, 11 and 6 of 24 cases), versus only 8 on the corresponding smear (25%). Of 11 samples derived from patients in partial or complete remission, all except one showed aberrant PC by five-colour FCM and/or by FISH on sorted PC, indicating the presence of minimal residual disease (MRD). Conclusion. FISH on flow sorted PC improves the detection of chromosomal aberrations in plasma cell disorders and is very well applicable in the routine diagnostics of MGUS, SM and MM for prognostic evaluation. Importantly, using the combined approach of five-colour FCM and interphase FISH on subsequently purified plasma cells, we were able to detect MRD in almost all of MM patients in remission. We propose this approach as a valuable alternative for other MRD detection methods such as flow cytometry alone and allele-specific PCR.

0249 BORTEZOMIB TRANSIENTLY INHIBITS OSTEOCLAST ACTIVITY IN CELL CULTURE CONDITIONS IMITATING IN VIVO INTERTREATMENT TREATMENT

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Background. Bone disease induced by multiple myeloma (MM) leads to severe pain, high risk for collapse of vertebral bodies and fractures of the major weight-bearing bones. It is due to acute degradation of bone matrix by osteoclasts, not coupled with new bone formation by osteoblasts. MM-induced bone disease is currently treated with bisphosphonates, highly effective bone resorption inhibitors, which do not stimulate but rather inhibit bone formation. Furthermore bisphosphonates may cause renal damage and osteonecrosis of the jaw. Therefore,
it is important to reconsider the management of MM bone disease in long-term treatment. Recently, preclinical studies have reported that the proteasome inhibitor Bortezomib used for the treatment of MM patients can stimulate bone formation, and that in MM patients treated with Bortezomib serum levels of bone formation markers are increased. Aims. In this study, we have investigated whether Bortezomib may inhibit osteoclast activity. Methods. Osteoclasts were differentiated from pure populations of CD14+ cells with the cytokines M-CSF and RANKL for 6-7 days. Cells were treated with Bortezomib at different concentrations in a continuous mode. It has been reported that prolonged inhibition of proteasome activity may be toxic for any cell type and in vivo pharmacodynamic studies have shown Bortezomib to be a transient inhibitor of osteoclast function only as 5min after intravenous injection, displaying maximal inhibitory activity of the proteasome within 24 hours subsiding rapidly thereafter. Therefore, Bortezomib was also given intermittently to mimic the in vivo situation. Osteoclast differentiation and activity were assessed by measuring Tartrate-Resistant Acid Phosphatase (TRAcP) activity in the medium. Cell viability was monitored using CellTiter Blue measuring metabolic activity. To extend our observations to the clinical situation, serum levels of CTX-I, a bone resorption marker, were measured during the 3 days following therapeutic Bortezomib administration in a single patient. Results. Continuous treatment with Bortezomib at 4nM and higher concentrations proved to be highly toxic for differentiating osteoclasts (cultures in presence of M-CSF+RANKL) but also monocytes (cultures in presence of M-CSF only) during a 7-day culture. However, a 6-hour pulse treatment with Bortezomib every third day, was not toxic to primary monocytes, even at a concentration as high as 25nM and a culture period as long as 7 days. In this condition, TRACP activity of osteoclasts was strongly inhibited during the first 24 hours with Bortezomib (65% inhibition at 25nM Bortezomib) but the activity returned to the control level after 72 hours. In the patient serum, serum levels of CTX-I decreased during the first 48 hours after each Bortezomib injection (n = 5), and tended to increase again after 72 hours suggesting a partial recovery of osteoclast activity between each dose. Conclusions. Our results suggest that Bortezomib temporarily inhibits osteoclast activity in vitro and in vivo. This transient inhibition of osteoclasts could be an advantage compared to the more persistent inhibition of osteoclast activity by bisphosphonate since recent reports suggested that formation of new bone requires at least a transient activity of osteoclasts. Further clinical studies are warranted to validate our findings.

**0251**

A NOVEL IN VIVO ANIMAL MODEL FOR HUMAN MULTIPLE MYELOMA BASED ON BIOILUMINESCENCE IMAGING OF TUMOR CELL GROWTH

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Preclinical testing of new therapeutic strategies or new cytotoxic drugs for the treatment of multiple myeloma (MM) requires animal models that closely resemble human disease and that allow quantitative evaluation of the applied therapy. Here we present a novel in vivo MM model by engraftment with U266 or RPMI-8226/S cells, both of human origin, into RAG2g2 double knock-out mice (RAG2GC). These mice are immune deficient because they lack T-, B and NK cells and the mice easily accept human cells (van Rijn et al., Blood 2008, Rozemuller et al., 2004). In this model we introduce the use of luciferase gene marking of the MM cells and applying Bioluminescence imaging (BLI) in living animals for measuring the initial growth of the MM cells and the response to treatment. After intravenous injection of 2x10⁶ cells MM cells engraftment and outgrowth occurred in all mice but it was limited to the bone marrow compartment, thus resembling human MM. FACS analysis revealed the presence of human CD45, CD138 and CD38 positive myeloma cells in a variety of examined bone specimen. Infiltration into other organs was not observed. MM cells were transduced with a GFP- Firefly luciferase (fluc) fusion gene. When luciferase converts the substrate lucerin, photons are emitted that can be registered by using sensitive CCD cameras. The absolute number of photons that are produced correlates (in our experiment) with the local tumor mass. Mice were injected i.v. with GFP-fluc 2x10⁶ cells MM cells (U266 or RPMI8226/S) and then imaged weekly using BLI. Within 2 weeks after injection significant BLI signals were detectable. Per mouse 5-10 foci showed luciferase activity, predominantly in the pelvic region, skin, limbs and the spine. All mice were examined weekly with BLI. We observed that the amount of light produced at the various foci of tumor growth, within an individual mouse as well as in between mice, showed a comparable increase. After 9-12 weeks all mice were killed due to excessive tumor growth. Growth curves that were made on the basis of subsequent BLI images revealed exponential growth of the total tumor mass per mouse as well as for the individual foci of MM growth in each mouse. All curves show similar growth kinetics with an average population doubling time of approximately 5-6 days. The range in which tumor growth can be monitored with BLI (and as a consequence also the response to treatment) spans 3-4 decades. The BLI signals could post-mortem be confirmed by flow cytometry of GFP+ cells in affected bones. The major advantage of this model is the option for quantitative evaluation of the effect of a given treatment has on the tumor load. In conclusion, we have developed a novel in vivo model to study the characteristics of homing and outgrowth of MM and we show that it can be used for quantitative evaluation of the efficacy of the therapeutic intervention.
0252

RELATIVE QUANTIFICATION OF TUMOR ASSOCIATED ANTIGENS MAGE-A1 AND MAGE-A3 IN MULTIPLE MYELOMA

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Background. Multiple myeloma (MM) is a malignant plasma cell neoplasm that often is preceded by a common pre-malignant monoclonal expansion of plasma cells called monoclonal gammopathy of undetermined significance (MGUS). MGUS is reported to be present in 1% of the adult population and to progres to MM at a rate of 1% per year. MM is an incurable tumor characterized by clonal expansion of malignant plasma cells in the bone marrow. The MAGE genes encode antigenic peptides that are presented by HLA class I molecules and that are recognized on human tumors by T lymphocytes. They are activated in a variety of malignant neoplasma while remaining silent in normal tissues with the exception of testis and occasionally placenta. Presence of RNA transcripts encoding members of the MAGE gene family in myeloma tumor cells and cell lines has been documented. Aims. The aim of this study is to evaluate the possibility of using these genes as molecular markers of the progression of MGUS to multiple myeloma and the early relapse of the MM. This abstract covers our pilot and preliminary Results. Total of 50 samples from bone marrow were evaluated: 25 samples from myeloma patients, 8 samples of patients with early stage of MM who did not require treatment (smoldering MM 2x and stage IA 6x). 5 samples of MGUS patients, 9 samples of normal healthy donors served as control group. Total RNA was evaluated by RT-PCR and then by real-time PCR using FRET probes on the LightCycler instrument (Roche). For relative quantification we used GePDH housekeeping gene as external standard. As positive control we used myeloma cell line U266. Results. None from samples of 9 healthy donors did show expression of MAGE. Only 1 of 5 (20%) samples from MGUS patient showed expression of MAGE-A1. Five (62,5%) from 8 patients with early stage of MM (IA and smoldering) showed expression of MAGE. On the contrary 11 (44%) of 25 samples from MM patients showed expression of at least one gene MAGE-A1 or MAGE-A3 or both (7 cases). Summary/Conclusions. We have confirmed that expression of MAGE is not present in samples of healthy donors. There is an obvious correlation between expression of the MAGE genes and early-late stage of the disease as our preliminary evaluation confirmed the detection of low expression levels of MAGE-type mRNA in bone marrow from patients with MGUS and early stage of MM. It is possible that MAGE antigen monitoring may predict the evolution towards more advanced disease as well as this method should be used for monitoring minimal residual disease in patients with MM. The prospective evaluation is under way. The actual results covering total of 15-50 evaluated patients in conclusive groups will be presented. This work is supported by grant of the Ministry of Education, Czech Republic, LCO0607.

0253

POST RELAPSE AND OVERALL SURVIVAL OF PATIENTS WITH MULTIPLE MYELOMA THAT PROGRESSED AFTER DECEMBER 1998

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Background. The prognosis of multiple myeloma (MM) patients progressing after autologous BMT (ABMT) was documented to be poor, ranging between 14-18 months in various reports. Since December 1998, a variety of novel methods were introduced for the salvage therapy of MM, namely Thalidomide, reduced intensity allogeneic stem cell transplantation (RIST) and later on Bortezomibe and Lenalidomide. Aims. To evaluate the impact of the introduction of novel methods in progressing MM. Methods. We report the outcome of a non-selected group of MM patients that progressed from ABMT after December 1998 and were treated in our center. The treatment strategy for this group of patients was based on the nature and the risk score of the relapse, according to the following milestones: 1. Treatment only at clinical indication; 2. Thalidomide with or without steroids; A. As first line of salvage therapy. B. At relapse from RISTC in a combination with donor lymphocyte infusion (DLI); 3. RIST or ABMT; A. for consolidation of response in patients resistant to thalidomide (after an induction of response with platinum containing regimen) and/or with high risk responding relapse. B. At escape from thalidomide effect; 4. Bortezomibe and Lenalidomide: A. in patients resistant to or escaping from Thalidomide effect, with an attempt to consolidate response by high dose therapy with allogeneic or autologous stem cell support. B. At progression from RISTC that did not respond to DLI and Thalidomide. Results. 84 patients (pts) that their disease progressed after ABMT between December 1998 and May 2004 were enrolled. All patients were treated with Thalidomide as first salvage therapy at a clinical indication, followed by the various options according to the scheme. At a later stage, 32 patients underwent RIST (22 from related and 10 from unrelated donors) and 13 patients had an ABMT. 16 patients were treated with Bortezomibe and 8 patients received Lenalidomide, for further progression. The median interval from detection of progression to initiation of therapy was 5.5 months. Response rate to thalidomide + steroids was 59% with a median duration of response (for responders not transplanted immediately at response) being 15 months (the longest exceeding 5.5 years). Transplant related mortality in RISTC was 22%. The 3 years overall survival (OS) for all the patients that underwent RISTC is 42%, and for those transplanted at response 61%. The median OS rate from progression, of the entire group of 84 patients, is 39 months. The median OS from first ABMT in this group is 84.3 months. The introduction, since 1998, of novel tools for the treatment of progressing MM, significantly prolongs the post relapse and the overall survival of patients with MM that undergo ABMT as a part of the initial therapy.

0254

OPG/ RANKL SYSTEM IN MULTIPLE MYELOMA

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Background. According to the contemporary ‘convergent’ hypothesis the major osteoresorptive and antiresorptive factors converge to the system osteoprotegerin (OPG)/receptor activator of nuclear factor kB-ligand (RANKL) and influencing its delicate balance they affect osteoclast proliferation, activation and apoptosis. Clinical results concerning the importance of the system in myeloma bone disease (MBD) are controversial. Aims. To analyse the serum levels of OPG and RANKL in patients with multiple myeloma (MM) and their correlations with clinical stage (Durie et Salmon), degree of MBD (according to the Merlini scale) and basic parameters of disease activity. Methods. We studied 66 newly diagnosed patients with MM, 29 male, 37 female, median age±SD: 61,8±8,6; range: 45-81years. In I / II / III clinical stage were 13,7% / 33,3% / 53,0% of patients resistant to thalidomide (after an induction of response with ABMT as a part of the initial therapy), accord-
RANKL/OPG ratio was also significantly higher in MM compared to controls (0.123 ± 0.019 vs 0.055 ± 0.019; p<0.001). The highest levels of RANKL and RANKL/OPG ratio were found in patients with MBD grade 2-3: 0.589 ± 0.076 and 0.185 ± 0.03 respectively. RANKL and RANKL/OPG ratio correlated strongly with clinical stage, MBD, bone marrow plasmacytosis, LDH and β2-microglobulin in the group without RF (Table). No correlation was found between RANKL, RANKL/OPG and the immunological variant, serum levels of Ca, creatinin, albumin, haemoglobin and albumin.

Conclusions. Our data show that OPG, RANKL and RANKL/OPG ratio are important clinical markers of MBD and a future target for therapeutic intervention. Increased OPG levels in patients with RF are most probably a result of compensatory reaction to the increased bone resorption, rather than to the reduced glomerular filtration.

0255 SAFETY AND EFFICACY OF BORTEZOMIB FOR RELAPSED/REFRACTORY MULTIPLE MYELOMA IN DAILY ONCOLOGY PRACTICE

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Background. Bortezomib is a novel first-in-class anti-cancer agent, a proteasome inhibitor. Several publications reported on the safety and efficacy of bortezomib in the treatment of relapsed/refractory multiple myeloma in clinical trials. Complementary data on the experience with bortezomib in daily oncology practice are needed. Aim. Evaluate the safety and efficacy of bortezomib in the treatment of relapsed/refractory multiple myeloma in routine clinical practice. Methods. Patients having undergone ≥2 prior lines of therapy were treated within a Compassion-ate Use Programme proposing 6 cycles of bortezomib. While treatment modalities were at physician’s discretion, the Programme recommended 1.3 mg/m 2 v. bortezomib on days 1, 4, 8 and 11 of each 21-day cycle. Addition of oral dexamethasone (20 mg the day of and the day after bortezomib administration) was recommended after 2 or 4 cycles in case of PD or SD, respectively. Post-hoc safety and efficacy analysis of patient records was performed using predefined criteria. Best response achieved was evaluated by the attending physician or by marker (M-protein or light chain) nadir level. Responders were patients with at least MR (a decrease ≥25% in marker level vs. baseline). CR/nCR was defined by physician’s criteria or by a decrease ≥95% in marker level vs. baseline. Patients that received <2 cycles and irregular injections were excluded from the main analysis. However, a response analysis on an intention-to-treat (ITT) basis also included these patients. Results. Eighty-eight patients entered the Programme involving 62 oncologists/haematologists. Data from 5 patients were unavailable. Main analysis focused on 69 patients and ITT response analysis included 83 patients. Median patient age was 66 years (44-86), median time since diagnosis was 4 years (0.5-14) and median number of previous treatments was 3 (2-6). Median number of bortezomib cycles at data collection was 4 (2-19) and 37.9% of patients were co-treated with corticoids during the Programme. In 73.9% of patients (51/69) at least an MR was observed (61.4% in the ITT analysis). In 57.7% of cases, this response occurred within the first cycle and in 95.5% within 3 cycles. Best response was achieved within the first cycle in 11.1% of patients and within 3 cycles in 68.9%. In the other responders, continuation of treatment improved the quality of response. Eighteen weeks after treatment initiation, corresponding to the anticipated duration of the Programme, 76.2% of the responders were still responding (≥2MR). At the time of data collection, median 4.75 months (0.5-15) after last bortezomib injection, 44.9% of patients (51/69) were still responding (37.3% of ITT patients), according to the physician’s evaluation. The most frequently reported adverse events were peripheral neuropathy (54.3%), thrombocytopenia (29.9%), diarrhoea (25.9%) and fatigue (22.4%). Among the cases of peripheral neuropathy, 65.2% were due to aggravation of a pre-existing condition. No cases of bortezomib-related haemorrhage were reported. Conclusions. The use of bortezomib in daily clinical practice resulted in encouraging high response rates with a predictable adverse event profile in patients with relapsed/refractory multiple myeloma. Efficacy and safety data were similar to those reported in clinical trials.

0256 MULTIPLE MYELOMA (MM) IN BRAZIL: CLINICAL AND DEMOGRAPHIC FEATURES AND THE UTILITY OF ISS IN 1,017 PATIENTS, MOSTLY WITH ADVANCED DISEASE

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Purpose. No large studies have described the features of MM in Brazil. Our aim was to characterize the demographic and clinical features of patients with MM treated at tertiary care centers, and to assess the predictive accuracy of the International Staging System (ISS) in such patients. Methods. 15 hematology centers provided information on patients diagnosed between 1998 and 2004, whose data were obtained from institutional charts and entered on a web-based system designed for the study. Chi-square tests were used for proportions, and survival was analyzed by the Kaplan-Meier method and log rank tests. Results. 1,017 patients (49.4% female), with ages ranging from 28 to 94 (median, 60.8 years), were evaluated. Race was white/mixed in 82.5%, black in 16.6%, and Asian in 0.9%. In 857 (84.2%) cases with immunoglobulin (lg) isotype, it was IgG in 52%, IgA in 18.6%, light chain in 9.9%, non-secretory in 3.4%, and IgM in 0.3%. Bone lesions were present in 64.7% of patients, and Durie-Salmon staging (DSS) was I in 6.5%, II in 16.4%, and III in 77.1% of cases. At the end of February, 561 patients (35.5%) had died. Median follow up for the entire sample was 20.2 months, and 26.8 months for surviving patients. There was a significant difference between the overall survival (OS) of patients with DSS I, II and III (p<0.001), but there was considerable overlap between the curves for stages I and II. Median OS for DSS I, II and III was not reached, 65.7, and 49.3, respectively. Among 934 patients with complete data, ISS category was I in 134 (14.3%), II in 585 (62.5%), and III in 217 (23.2%). The median OS was not reached, 57.5 and 24.6 months for these three groups, respectively (p<0.001). After 5 years, the estimated OS for patients with ISS I was 68%, 257 (25.3%) patients underwent high-dose chemotherapy (HCT), and had a median OS of 77.2 months, compared with 39.4 months for those not receiving HCT (p<0.001). Other significant factors for OS in univariate analyses were hypercalcemia (p<0.001) and elevated LDH (p=0.028). Hypercalcemia was found in 10.7% of patients with ISS I, 28.3% in ISS II, and 54.0% in ISS III (p<0.001). Inspection of the OS curves shows ISS to be more useful than DSS in this sample with mostly advanced disease, with better segregation of the groups and nominally smaller P values than seen with DSS. Conclusion. This retrospective study confirms the prognostic utility of ISS, and suggests that ISS is more useful as a prognostic indicator in a sample of patients diagnosed in late stages of the disease, as determined by the DSS.

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EVALUATION OF 4 STAGING SYSTEMS IN 470 MYELOMA PATIENTS: VALIDATION OF THE SUPERIOR PROGNOSTIC SIGNIFICANCE OF THE INTELLIGENT STAGING SYSTEM

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Background. The wide range in survival rates in multiple myeloma (MM) establishes the necessity for a staging system with prognostic reliability. For more than 30 years, the Durie-Salmon Staging System (DSSS) has been recognized and subsequently employed by Bataille et al., the South West Oncology Group (SWOG) and most recently the International Myeloma Working Group (IMWG), in attempts to develop simpler staging systems with a stronger prognostic impact. Aims: To evaluate and compare the prognostic significance of these 4 staging systems in a large number of previously untreated MM patients. Methods. Between January 1989 and January 2006, 470 consecutive patients were diagnosed with MM in our department. Ninety-two (19.6%) patients received high dose therapy followed by autologous transplantation and the rest 378 (80.4%) were treated with conventional chemotherapy. All patients were classified according to the following staging systems: 1) DSSS. 2) Staging System of Bataille et al. (BSS). Stage I: β2m <6 mg/L and alb ≥3g/dL. Stage II: β2m ≥6 mg/L, and alb ≥3g/dL. Stage III: alb ≥5g/dL. 3) Stage System of the SWOG (SWSS). Stage I: β2m <2.5mg/L. Stage II: 2.5 mg/L ≤β2m <5.5 mg/L. Stage III: β2m ≥5.5mg/L and alb ≥3g/dL. 4) International Staging System (ISS) of the IMWG. Stage I: β2m <3.5mg/L and alb ≥5g/dL. Stage II: neither stage I nor III. Stage III: β2m ≥5.5 mg/L. Overall survival (OS) was estimated according to Kaplan-Meier method. Differences in survival were assessed using the log-rank test. Results. The distribution and median OS of the patients according to each staging system are displayed in Table 1. Classification according to DSSS, BSS and SWSS yielded a significantly heterogeneous distribution of our patients, with the majority being classified in stage III, I and II respectively. ISS achieved the most homogeneous patient distribution. There was no statistically significant difference (<0.05) in survival between stages II and IIIA of DSSS, II and III of BSS, as well as between stages III and IV of SWSS. ISS alone yielded significant difference (p<0.0001) in survival between all three stages. Conclusion. Our study confirms the superiority of ISS over DSSS and previous prognostic classifications based on the combination of β2m and albumin. ISS proved to be a simple, reproducible alternative with high prognostic power, definitely able to gain wide clinical applicability.

Table 1.

<table>
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<tr>
<th>Stage</th>
<th>N (%)</th>
<th>OS months (95% CI)</th>
<th>N (%)</th>
<th>OS months (95% CI)</th>
<th>N (%)</th>
<th>OS months (95% CI)</th>
<th>N (%)</th>
<th>OS months (95% CI)</th>
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<td>I</td>
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<td>21 (62-78)</td>
<td>270</td>
<td>53 (44-62)</td>
<td>37</td>
<td>73 (15.5)</td>
<td>273</td>
<td>76 (67-85)</td>
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<tr>
<td>II</td>
<td>131</td>
<td>21 (62-78)</td>
<td>270</td>
<td>53 (44-62)</td>
<td>37</td>
<td>73 (15.5)</td>
<td>273</td>
<td>76 (67-85)</td>
</tr>
<tr>
<td>III</td>
<td>301</td>
<td>47 (37-57)</td>
<td>79</td>
<td>25 (21-31)</td>
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<td>45 (41-49)</td>
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OSTEONECROSIS OF THE JAW IN PATIENTS WITH MULTIPLE MYELOMA TREATED WITH BISPHOSPHONATES: A SINGLE CENTER EXPERIENCE

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Background. Bisphosphonate (BP)-associated osteonecrosis of the jaw (ONJ) is a new and distinct clinical entity. Cases of ONJ associated with the administration of BPs, that is characterized by dehiscence of the oral mucous membranes and exposure of necrotic underlying mandible or maxilla, were first reported in 2003. Aims. To study retrospectively a large number of multiple myeloma (MM) patients treated with BPs, in order to estimate the incidence and identify possible risk factors for the development of ONJ. Methods. Administration of BPs was initiated in our department in 1991. A review of the medical records of all patients diagnosed with MM since 1991 was performed. We evaluated the type of BP administered, the time of exposure to BPs and the cases of ONJ. The diagnosis of ONJ was based on the presence of symptoms and signs of intraoral bone necrosis, the findings of panoramic x-rays and the results of bone biopsies. Patients were divided into 3 groups according to the type of BP administered. Group A received pamidronate, Group B zoledronate and Group C pamidronate and zoledronate sequentially. The χ2 test was used for comparisons of proportions across levels of categoric variables. Mann Whitney U test and One Way ANOVA test were used to compare the median and mean time of exposure to BPs among groups respectively. Kaplan Meier method was used to estimate the actuarial risk of ONJ in each group. Differences were assessed using the log-rank test. Throughout the analysis a level of 5% was used to denote statistical significance. Results. Between 1991 and 2005, 303 patients with MM were diagnosed in our department. Bisphosphonates were administered to 254 (83.8%) patients with median time of exposure 15 months (4-77). Group A included 78 patients (30.7%), Group B, 91 (35.8%) and Group C, 85 (33.5%) with median time of exposure 10 (4-38), 12 (4-52) and 36 (6-77) months respectively. (pA,C <0.000, pB,C<0.000). Forty-nine (16.2%) patients did not receive BPs. Twenty eight cases (11.02%) of ONJ were observed among patients treated with BPs. None of the patients without exposure to BPs developed ONJ. The actuarial risk of ONJ after 18 months of administration was 3.7% for group A and 7.8% for group B (p=0.25). At 36 months the actual risk of ONJ was 37.2% and 12.4% for group B and C respectively (p=0.036). Conclusions. The incidence of ONJ in patients with MM treated with BPs is high. Time of exposure and probably the type of BP seems to contribute to the occurrence of ONJ.
Chronic lymphocytic leukemia and related disorders – Clinical/Experimental

0259
DETECTION OF RISK-IDENTIFYING MARKERS AND ADDITIONAL ABERRATIONS BY MULTIPLEX LIGATION-DEPENDENT PROBE AMPLIFICATION (MLPA) IN CHRONIC LYMPHOCYTIC LEUKEMIA
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B cell chronic lymphatic leukemia (B-CLL) is the most common form of leukemia in adults. Recently, deletions of ATM, TP53 and trisomy of chromosome 12 have been identified as unfavorable markers using interphase FISH. FISH analysis is labor-intensive, expensive and limited to the number of probes analyzed. We developed a robust method based on multiplex ligation-dependent probe amplification (MLPA) of target sequences for 40 different tumor-associated genes, including unfavorable risk-identifying deletions of ATM and TP53 and trisomy of chromosome 12. MLPA data of 53 cases with CLL and one case with follicular lymphoma (FL) were validated using conventional karyotyping and interphase FISH analysis, revealing high sensitivity and specificity of the assay for these risk-identifying mutations. DNA profiling using MLPA identified recurrent gain of TP53 and BCL2 (18q21.3), known targets in B-cell non-Hodgkin lymphoma (NHL). A recurrent deletion of CDKN2A/B locus (9p21) was found, that was associated with aggressive disease progression. A trisomy chromosome 19 was identified that went undetected by cytogenetics. MLPA confirmed trisomies of chromosome 12, which we demonstrated earlier. This method can be used for rapid analysis of known risk-identifying markers and detection of additional numerical cytogenetic imbalances in B-CLL.

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QUANTITATIVE GENE EXPRESSION ANALYSIS OF SURROGATE MARKERS FOR GENETIC RISK GROUPS AND SURVIVAL IN CLL
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Background. The genetic factors VH mutation status, V3-21 gene usage, and genomic deletions at 11q22-23 and 17p13 have been shown to be important prognostic markers in CLL. Given the high complexity of these analyses in the recent years several molecular surrogate markers have been developed according to the facility of routine prognosis assessment. Aims. To assess the value of potential surrogate markers for the prediction of genetic risk groups and survival. Methods. Real-time RT-PCR (RQ-PCR) of candidate genes was performed in a CD19-purified and a non-purified CLL cohort each comprising the relevant genetic subgroups. Immunofluorescence sensitivity analysis included 11q-, 17p- and 17p- mark- ers (ADAM29, ATM, CLL1U, DMD, GLO1, H51, KIAA0977, LPL, MGC9913, PDCD9, PEG10, SEPT10, TCF7, TP53, Vimentin, ZAP-70, ZNF2) identified in previous studies were investigated in the non-purified cohort of 102 CLL patients. Of these, 10 markers, either with an overexpression in non-CLL cells or an impact on survival or risk group prediction, were analyzed in the purified cohort of 112 cases. VH sequencing and FISH screening for genomic aberrations were carried out for all cases. Survival information was available for 80 (purified) and 88 cases (non-purified). Logistic regression was performed to test the predictive value of gene expression for genetic risk groups, Cox proportional hazards statistics for survival analysis. Results. The genetic risk groups in both cohorts showed the expected correlation with survival with significantly shorter survival of VH unmutated, 17p-, and 11q- cases indicating a representative composition of the cohorts under study. In non-purified cases, the best predictive marker for VH status was LPL (p=0.001). While no reliable predictive markers were identified for V3-21 usage or 17p-, ATM expression was predictive for 11q-. In sur- vival analysis including all candidate genes, TCF7 (p=0.001) and KIA0977 (p=0.016) were of prognostic value. In multivariate survival analysis including candidate gene expression and the genetic risk factors as vari- ables, only 17p- remained as a significant parameter. In the purified cohort, significant markers (p<0.05) for genetic risk groups were: ZAP70, LPL, and TCF7 for VH mutation status (TCF7 expression associated with mutated VH); SEPT10, ZAP70, and ADAM29 for V3-21 usage; ATM and TCF7 for 11q- (both with a negative association); ZNF2 for 17p- (negative association). In survival analysis including the expression of all candidate genes, only TCF7 was identified as a significant factor. In contrast to ZAP70, which was of borderline significance (p=0.061), TCF7 expression was positively correlated with survival times. In multivari- ate analysis, the parameters 17p-, 11q-, V3-21 usage, TCF7 and ZAP70 expression were identified as independent prognostic factors. Summary. Several results of this study could not be reproduced in unpurified cases strongly arguing for a tumor cell selec- tion prior to expression analysis. In purified cases, ZAP70, LPL, and TCF7 were the best predictors for VH mutation status. Additional mark- ers such as ATM and ZNF2 may help to identify genomic risk groups such as 11q- and 17p-. Multivariate survival analysis suggests TCF7 as a strong survival predictor and points to a pathogenic role for this gene in CLL.

0261
DISTINCT EXPRESSION LEVELS OF NOXA IN PERIPHERAL VERSUS LYMPH NODE CHRONIC LYMPHOCYTIC LEUKEMIA CELLS ARE LINKED WITH SURVIVAL CAPACITY
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Background. The relentless accumulation of chronic lymphocytic leukemia cells is presumed to derive from proliferation centers in lymph node and bone marrow. To what extent the properties of these leukemic cells are linked with the often stated characteristic anti-apoptotic phe- notype of CLL is unknown. Recently, we have described that in periph- eral blood CLL samples, aberrant apoptosis gene expression was not limited to protective changes but also included increased levels of pro- apoptotic Noxa and Bmf. The functional consequence of this finding is not known, nor whether this aberrant apoptosis gene profile is also present in CLL proliferation centers. Aim. To perform a functional compar- ison of apoptosis gene profiles from peripheral blood CLL versus lymph node proliferation centers. Methods. Immunofluorescence microscopy, RT-Multiplex-Ligation-dependent Probe Amplification (RT-MLPA), Western blot, Transfection, RNA interference. Results. Lymph node material from 9 B-CLL patients and peripheral blood samples from 16 B-CLL patients were included. All B-CLL expressed CD5, CD23 and CD19 or CD20. Over 90% of the lymph nodes consisted of lymphocytes. Ki67+ cells were either scattered throughout the lymph nodes or in follicle-like structures. RNA samples were subjected to the RT-MLPA procedure which monitors expression of 34 apoptosis genes. Apart from expected differences in survivin and Belxi, the most prominent distinct- ion with peripheral CLL cells was the generally low levels of Noxa in lymph node samples. A reduction in Noxa RNA and protein levels could also be obtained by in vitro stimulation of peripheral blood CLL with CD40. Direct manipulation of Noxa protein levels was achieved by pro- teasome inhibition in CLL and via RNAi in model cell lines. In all these instances, the viability of the cells was directly linked with Noxa levels. Conclusions. These data indicate that spontaneous apoptosis of peripheral CLL cells in vitro is linked with high Noxa levels. We propose that suppression of Noxa in the lymph node contributes to the persistence of CLL, and that therapeutic targeting of Noxa might be beneficial. This work was supported by the Dutch Cancer Foundation (DCF).

0262
SIGNIFICANT CORRELATION BETWEEN OBJECTIVE RESPONSES AND EXPOSURE TO HUMAX-CD20 IN CHRONIC LYMPHOCYTIC LEUKEMIA
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Background. The fully human monoclonal IgG1 antibody HuMax- CD20 targets a novel epitope of the CD20 molecule on B-cells. HuMax-CD20 stops growth of engrafted B-cell tumors in SCID mice more effi-
ciency than Rituximab and i.v. infusion of HuMax-CD20 in cynomol-gus monkeys leads to profound, long lasting, dose-dependent B-cell depletion. Aim. The objective of the present trial was to establish the safety, efficacy and the pharmacokinetics of HuMax-CD20 in patients with chronic lymphocytic leukemia. Methods. Data are presented from an open label, dose-escalation, multicenter phase I/II clinical trial. 3 cohorts were in accordance with the prerequisite of 27 (C) patients with relapsed or refractory chronic lymphocytic leukemia (B-CLL) received 4 weekly i.v. infusions of HuMax-CD20 and will be followed for 12 months. The first infusion was 100 mg, 300 mg and 500 mg in cohort A, B and C, and the following 8 infusions were of 500, 1000 and 2000 mg, respectively. Patients were premedicated with oral acetaminophen and i.v. antihistamine and received i.v. glucocorticoids before first and second infusions. The endpoints were B-cell depletion, adverse events, objective response according to the NCI working group guidelines for CLL, time to progression, duration of response, time to next anti-CLL treatment, and pharmacokinetics. Results. Median age was 61 years; median time since diagnosis was 6.3 years. Maximum tolerated dose was not reached. All patients in the highest dose group had pronounced reduction of the leukemic CD19+CD5+ cell counts. Objective response rate was 46% (12 of 26 evaluable patients in cohort C) with 2 nPR and 10 PR. By analyzing pharmacokinetic parameters, a statistically significant increased AUC were demonstrated in responders (median: 1256 µg/mL*h, range: 318-1820 µg/mL*h; p<0.01) compared to non-responders (median: 949 µg/mL*h, range: 540-1260), p=0.011. Similar significant differences were found for Cmax and Cmin. Conclusion. This preliminary analysis of data from the first 33 CLL patients treated with HuMax-CD20 demonstrated significant depletion of CD19+CD5+ cells and provided an indication of clinical efficacy that correlates with the exposure to HuMax-CD20. These data encourage further development of HuMax-CD20 in CLL.

CD40 LIGATION SENSITIZES P53 dysfunctionAL CCLs TO CHEMOTHERAPY INDUCTED APOPTOSIS VIA THE PT3 PATHWAY

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CD40 activation of chronic lymphocytic leukemia (CLL) cells enhances their capacity to induce an immune response. Although CD40-activation has been shown to enhance sensitivity to both cytotoxic T cell mediated killing and death receptor mediated apoptosis, its effect on chemotherapy is much less clear. We showed recently that CD40 activation of CLL cells resulted in induced expression of pro-apoptotic factors like death receptors, p21 and the BH3-interacting-domain death agonist (Bid), even in CLL cells with dysfunctional p53. Since the effect of fludarabine is highly mediated by p53 dependent response genes we hypothesized that CD40 activation could sensitize p53 dysfunctional CLL cells to fludarabine mediated apoptosis. In ex vivo studies, we show that stimulation of CLL cells by co-culture with CD154-expressing cells induced leukemia-cell expression of p73, a p53-related transcription factor that is regulated by the c-Abi tyrosine kinase in both p53 functional and dysfunctional cases. Transduction of CLL cells with an adenovirus encoding p73 also induced Bid. Next we showed that p53-dysfunction-al CLL cells resistant to fludarabine treatment could be sensitized to fludarabine upon CD40 ligation or p73 transduction. This phenomenon could be suppressed by specific c-abl inhibition through imatinib treatment. These results demonstrate that CD40 ligation may sensitize leukemia cells not only to extrinsic but also intrinsic apoptotic stimuli via a c-Abi-dependent pathway and that CD40-based therapy may be helpful in overcoming the resistance of p53-dysfunctional CLL to anti-cancer therapy.
In conclusion, the rapid, selective, p53-independent cytotoxic action of PTI 112 suspended in saline indicates a biologic effect of the agent, suggesting that this agent may be of value in the treatment of CLL patients resistant to conventional regimes. These data also suggest that further investigation of natural products that induce ROS may be of potential therapeutic use for the treatment of B-CLL.

0266
SMALL MOLECULE Pan-BCL2 FAMILY INHIBITOR OBATOCLAX (GX15-070): FINAL RESULTS OF A SINGLE AGENT PHASE 1 TRIAL IN PATIENTS WITH REFRACTORY CHRONIC LYMPHOCYTIC LEUKAEMIA (CLL)

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Background. Obatoclax is a synthetic small molecule which inhibits the binding of the antia apoptotic proteins bcl-2, bcl-xl, bcl-w and mcl-1 to the proapoptotic proteins bax and bak. It shows broad single agent cytotoxicity in cancer cell lines in vitro and in vivo and in human B-CLL cells ex vivo. Bcl-2 family proteins are universally overexpressed in CLL in which accumulation of malignant cells is thought to be the direct result of the consequence inability to undergo apoptosis. Aims. determine safety profile, recommended phase II dose, pharmacokinetic and pharmacodynamic profile of obatoclax in refractory CLL. Methods. accelerated dose escalation of 1-3 h infusions every 3 weeks with single intra-patient escalation at doses ranging from 3.5-14 mg/m2 administered over a 1 hr infusion and 13 patients at 20-40 mg/m2 over a 3 hr infusion. Median age was 61 (range 46-76). Rai stage was III-IV in 19 and median number of prior therapies was 4 (range 2-10). The most frequent adverse events (AE) have been somnolence (40%) and fatigue (34%). Dose-limiting toxicities have been Grade 1 (40%) or 2 (19%) and euphoria Grade 1 (47%) or 2 (9%) occurring during or shortly following the infusion. Other AE’s reported in ≥25% of patients were transient O2 desaturation (25%), AST increase (34%) and fatigue (34%). Dose-limiting toxicities have been Grade 3 infusional neurological events such as somnolence, ataxia and dysphoria. Doses ≥10 mg/m2 were associated with a significant increase in activated bax/bax hetero oligomers sustained for up to 8 hrs at the higher doses evaluated. An early release of ODNA occurred 1-6 hrs after the start of the infusion. A secondary increase occurred with a noticeable lag time from the peak plasma GX15-070 concentration (24 to 168 hrs after the start of the infusion). There was a correlation between peak plasma ODNA concentration (median = 400 range 0-4358 AU/mL) and dose (threshold effect at 14 mg/m2) as well as AUC (maximum ODNA 2x baseline if AUC≥180 ng/hr/mL vs. 15 x baseline when AUC≥180 ng/hr/mL; p<0.015). 18/25 patients showed reduction of peripheral lymphocyte counts (mean of 29%). Best clinical responses assessed by CLL Work-Shop Criteria so far are: unconfirmed PR in 1. In addition, 4/14 patients with baseline platelet count <100,000/mm3 showed sustained elevations of platelet counts by ≥50% including two patients improving from 70,000 to 144,000/mm3 and 47,000 to 105,000/mm3; 3/11 patients who were anemic at baseline showed sustained elevations of Hb from 8.7 to 10.6 g/dL, 7.9 to 13.9 g/dL and 8.7 to 9.8 g/dL, the latter two achieving transfusion independence. Conclusions: single agent obatoclax exhibits dose dependent and it seems to be the most important prognostic factor in B-CLL. Identifying patients with a more aggressive clinical course was reported at 34% (30-41) of patients. Conclusions. At diagnosis of CLL, even within a group of patients with a high clinical response rate, ex vivo drug sensitivity can be used to identify a proportion of patients with a significantly poorer probability of clinical response. TRAC results predict better for patient response to fludarabine (± cytosphamide) than for response to chlorambucil.

Table 1. Comparison of ex vivo drug sensitivity with subsequent patient response (numbers of patients).

<table>
<thead>
<tr>
<th>Trial arm</th>
<th>No. Response</th>
<th>No Response</th>
<th>Response</th>
<th>No Response</th>
<th>Odds ratio 2P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chl</td>
<td>210</td>
<td>141</td>
<td>45</td>
<td>9</td>
<td>5.2 (2.1-13) 0.0004</td>
</tr>
<tr>
<td>Flu+Flucy</td>
<td>232</td>
<td>194</td>
<td>20</td>
<td>4</td>
<td>13.4 (10.113)&lt;0.0001</td>
</tr>
</tbody>
</table>

Total 442 335 65 13 29 11.5 (5.73)<0.0001

0268
INTRACELLULAR CYTOKINE EXPRESSION BY B AND T CELLS DIFFERS IN ZAP-70 POSITIVE AND ZAP-70 NEGATIVE B-CELL PATIENTS

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Background. Changes in cytokine network between malignant cells and residual T lymphocytes may be responsible for accumulation of malignant cell clone and for immune abnormalities in B-cell chronic lymphocytic leukemia (B-CLL). B-CLL is the most frequent type of adult leukemia in Western countries and it seems to be a heterogeneous disease with a highly variable clinical course and prognosis. Recently the role of ZAP-70 (zeta associated protein, a member of the Syk-ZAP-70-protein tyrosine kinase family) as a surrogate marker for IgVH mutation identifying patients with a more aggressive clinical course was reported and it seems to be the most important prognostic factor in B-CLL. The aim of this study was to try to determine whether cytokine expression pattern changed according to ZAP-70 expression. We assessed the expression of IL-2, IL-4, IFN-γ and TNF-α by CD19 and CD3 cell subsets in group of ZAP-70 positive and ZAP-70 negative B-CLL patients. Methods. We analyzed the blood samples obtained from sixty newly diagnosed, untreated B-CLL patients. Peripheral blood lymphocytes were isolated and stimulated by PMA and ionomycin in the presence of BrdU A to assesses intracellular cytokine expression. Then combined membrane and intracytoplasmic staining procedures were performed using fluorochrome-conjugated monoclonal antibodies. All samples were stained for ZAP-70 protein expression by CD19+CD5+ cells. The multi-color flow cytometry technique was used to analyze labeled cells. The data were shown as percentage of analyzed cell subset and mean fluorescence intensity (MFI) indicating the level of cytokine expression by particular cell. Results. The mean percentage of CD3 cells expressing all analyzed cytokines was significantly higher in ZAP-70 positive in
comparison to ZAP-70 negative group. Such a difference was observed in MFI values, however it was not statistically significant. The mean percentage of CD19/TNFα cells was significantly higher, while the percentage of CD19/IL-2, CD19/IL-4 and CD19/IFNγ was lower in ZAP-70 positive than ZAP-70 negative patients. There was no significant difference between ZAP-70 positive and ZAP-70 negative group as far as MFI was concerned. Conclusion: TCLVα and TCLVγ express an association between ZAP-70 and cytokine expression in B-CLL. The more aggressive course of disease is connected with higher capability of T cells in production of cytokines responsible for disease pathogenesis. Such a connection is also observed in production of TNFα by malignant B lymphocytes. Our results may approve the role of ZAP-70 expression by malignant cells as a good prognostic marker for B-CLL.

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0269
ACCUMULATION OF T-CELL WITH EXTREMELY SHORT TELOMERES IN T-CELL PROLIFEROPHICTIC LEUKEMIA (T-PLL)
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Background. T-cell proliferopihctic leukemia (T-PLL) is a rare aggressive lymphoproliferative disease characterized by the expansion of a T-cell clone derived from immuno-compotent post-thymic T-lymphocytes. Important mechanisms involved in expansion of human malignant cells are reactivation of telomerase, an enzyme complex, which is able to compensate the loss of telomere repeats by cell division, and maintenance or elongation of telomere length. Aims. Our aim was to investigate the role of telomeres in patients with T-PLL. Methods. We measured telomere length by automated multicolor flow-FISH and telomerase activity by telomeric repeat amplification protocol in subsets of peripheral blood leukocytes from 10 newly diagnosed or relapsed patients with sporadic T-PLL. Results. The average telomere length in the clonal T-cells of all samples analyzed was extremely short (mean±SD: 1.6±0.6 kb) compared to the non-clonal T-cells (5.5±0.8 kb; p=0.012). The average telomere length for B-cells in these patients was 6.4±0.7 kb, n=5. Telomere length values of the clonal T-cells were all below the 1st percentile of telomere length values observed in T-cells from healthy aged-matched controls whereas non-clonal T-cells and B-cells fell between the 10th and 90th percentile of the normal distribution. In addition, we performed follow-up measurements of telomere length in one patient over a period of 18 months. Surprisingly, telomere length remained stably short at 1.0 kb±0.6 kb in the clonal T-cells without further telomere loss. No cell doublings indicative of fused or bridged chromosomes and telomere dysfunction were observed. Although telomerase is necessary to elongate or stabilize short telomeres levels of telomerase activity in the clones were higher than in normal controls. Conclusions. This is the first report of extremely short telomeres in clonal T-cells of patients with sporadic T-PLL. Our results are compatible with extensive proliferation of the clone. Most likely telomerase activity in T-PLL is sufficient to stably maintain extremely short telomeres and allow their clonal expansion. Current studies are aimed at exploring telomerase inhibitors to inhibit the proliferation of T-PLL cells and at the role of the very short telomeres in these cells regarding genomic instability and cytogenetic aberrations.

0270
INTRACLONAL DIVERSIFICATION OF IMMUNOGLOBULIN LIGHT CHAIN VARIABLE REGION GENES IN CHRONIC LYMPHOCYTIC LEUKEMIA
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Analysis of somatic mutations in immunoglobulin heavy chain variable region (IGHV) genes in various types of B cell malignancies, including chronic lymphocytic leukemia (CLL), has demonstrated frequent intraclonal heterogeneity, indicating ongoing mutational activity. We recently showed that CLL light chain repertoire is skewed and characterized by CLL-biased features and also provided evidence for the complementary role of light chains in antigen recognition by CLL malignant cells. In the present study, we evaluated the intraclonal diversity status of IGKV/IGLV genes in 32 CLL cases; 25 cases expressed IgM/IgD, whereas non-clonal T-cells and B-cells fell between the 10th and 90th percentile of telomere length values observed in T-cells from healthy aged-matched controls whereas non-clonal T-cells and B-cells fell between the 10th and 90th percentile of the normal distribution. In addition, we performed follow-up measurements of telomere length in one patient over a period of 18 months. Surprisingly, telomere length remained stably short at 1.0 kb±0.6 kb in the clonal T-cells without further telomere loss. No cell doublings indicative of fused or bridged chromosomes and telomere dysfunction were observed. Although telomerase is necessary to elongate or stabilize short telomeres levels of telomerase activity in the clones were higher than in normal controls. Conclusions. This is the first report of extremely short telomeres in clonal T-cells of patients with sporadic T-PLL. Our results are compatible with extensive proliferation of the clone. Most likely telomerase activity in T-PLL is sufficient to stably maintain extremely short telomeres and allow their clonal expansion. Current studies are aimed at exploring telomerase inhibitors to inhibit the proliferation of T-PLL cells and at the role of the very short telomeres in these cells regarding genomic instability and cytogenetic aberrations.

Background. Patients with chronic lymphocytic leukemia (CLL) relapse even after aggressive therapies and stem cell transplantation. As the therapeutic goal today is to clear off the tumor cell burden as much as possible (by stem cell transplantation or intensive chemotherapy) and to provide highly sensitive assays for minimal residual disease (MRD) evaluation and monitoring are needed. At present, many patients with not only germline IgVH sequences, but also with hypermutated IgVH genes are being treated, with the need for a sensitive and specific MRD monitoring. The original notion of MRD follow-up in CLL was based on the usage of IgH-generecognizing Tcell hybridoma clones or single B cells. Due to the vast diversity of B-clonal rearrangements to be detected, the original idea has been challenged and the methodology should be modified. Aims. Since the hypermutation process does not restrict itself to the VH segments only and might affect the JH segment as well, the molecular tools for the monitoring of B-CLL clonal rearrangements must be versatile enough to allow for the detection and quantitation of virtually any sequence possible. Moreover, the technique must meet the criteria for high sensitivity and specificity. We present here a novel methodology for MRD monitoring in CLL, based on LNA technology (Locked Nucleic Acids) and quantitative Real-Time PCR. Methods. Thirty-nine patients with the diag.
TELOMERE LENGTH IS A PROGNOSTIC FACTOR STRONGER THAN VH-MUTATIONAL STATUS IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA (B-CLL)

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Background. Telomere restriction fragments (TRF) length has a prognostic impact in B-CLL. Some studies suggest that this is a mere reflection of its association to VH-mutational status (VH-MS). However, the relative value of these two parameters has not been clearly defined, particularly in cases in which they are discordant.

Aim. To compare, in a large population of B-CLL patients, the prognostic impact of TRF length and VH-MS, in terms of overall survival (OS), time to first treatment (TTFT) and progression free survival (PFS).

Patients and Methods. 184 B-CLL patients have been analyzed for TRF length and VH-MS. All samples were taken before treatment start. Males were 118, females 66. Median age was 62 years (range 34-87). According to Binet staging system, 117 were stage A, 34 B and 33 C. Cytogenetics, CD38 and ZAP-70 expression were available in 80% of patients. Median follow-up was 36 months (range 6-290). Eighty-seven patients have been already treated.

Methods. TRF length was evaluated by Southern blot and VH-MS by direct sequencing. The standard cut-off of 2% deviation from any germ line VH sequence was employed to define VH-MS. Survival analyses were performed using the Kaplan-Meier method. Cox multiple regression was used to analyze the independence of the following potential prognostic parameters: sex, age, Binet stage, CD38 and ZAP-70 expression, cytogenetic features, VH-MS and TRF-length.

Results. Median TRF length was 6000bp (range 1465-14837bp). There was no correlation between TRF length and patient age, sex or stage. TRF length had a major impact on prognosis with best results observed with a cut-off of 4250bp. Patients with TRF<4250bp had a worse outcome than patients with TRF>4250bp (median OS: 85 vs 269 months, p<0.001; median TTFT: 21 vs 63 months, p<0.0001; median PFS: 12 vs 36 months, p<0.0001). VH-MS analysis was successful in 91%. Overall, discordance between VH-MS and TRF length was observed in 16% of patients. Discordance was common among VH-unmutated patients (58%) but rare among VH-mutated patients (6%). Discordant and concordant patients could not be distinguished based on VH usage or degree of homology (H) to the germline IgH sequence (i.e. H=100% vs H=100% and >99% vs H=99% and >98%). In addition they could not be distinguished based on stage, cytogenetics, CD38 and ZAP-70 expression. The 24 discordant patients with VH-unmutated status and TRF length<4250bp had a clinical outcome that was significantly different from VH-unmutated patients with TRF length>4250bp (median OS: 83 vs 215 months, p<0.05 and median PFS: 12 vs 33 months, p<0.05) and similar to that of VH-mutated patients (median OS 269 months and median PFS 54 months, p=n.s.). Finally, the multivariate analysis indicated that TRF length and Binet stage were the most important prognostic indicators in B-CLL.

Conclusions. Our data demonstrate that: 1) TRF length is a major prognostic indicator in B-CLL in terms of OS, TTFT and PFS; 2) when discordance exists between VH-MS and TRF length the latter better predicts outcome.

HIGHLY SENSITIVE DETECTION OF MINIMAL RESIDUAL DISEASE IN B-CELL CHRONIC LYMPHOCYTIC LEUKAEMIA BY INTERPHASE FLUORESCENCE IN SITU HYBRIDIZATION ON FLOW SORTED CELLS


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Background. The introduction of new therapeutic agents such as fludarabine and alemtuzumab, with or without autologous or allogeneic stem cell transplantation, has improved outcome. Nevertheless, complete remissions rates in B-cell chronic lymphocytic leukaemia (CLL) remain high. Progression rates after several years of therapy are only in the range of 5-10% which suggests that CLL cells in complete remissions are not eradicated completely. It is not clear whether residual low numbers of malignant cells (minimal residual disease (MRD)) have any impact on outcome and whether these cells are present in 100% of complete responders.

Aim. We have developed and validated a combined approach to assess MRD in CLL using fluorescence-activating...
cell sorting (FACS) and interphase fluorescence in situ hybridization (FISH) for the detection of numerical chromosomal aberrations that occur in up to 80% of CLL cases. Methods. CLL cells were purified from the peripheral blood of CLL patients by FACS-Aria (BD, US) based on the CD19+CD5+ co-expression, with a purity of > 95%, as assessed by microscopy and by reanalysing with flow cytometry. These CLL cells were then stained with deletion 11q (ATM) or deletion 13q14 in > 95%, by using dual colour FISH. Peripheral blood samples from normal individuals were spiked with the purified CLL cells with dilutions of 10^−10^−6 white blood cells (WBC). WBC from these spiked samples were subsequently labelled with CD19 and CD5 moAbs and analysed by FACS. CD19+CD5+ cell fractions were purified by FACS-Aria and analysed for deletions 13q14 or deletion 11q. Results. FISH detection of the specific chromosomal aberration in CD19+CD5+ purified cells allowed discrimination of CLL cells from normal precursor B-cells. Reproducible positive results, above cut-off levels of the probe, were demonstrated in all dilutions up to 10^−6^ or 10^−1^.

Quantification was feasible using the percentage of CD19+CD5+ cells and the percentage of aberrant purified cells. Conclusions. This approach for the detection and quantification of MRD in CLL reaches a sensitivity at least as high as and even higher than other methods, such as four-color flow cytometry or quantitative allele-specific PCR. It can be used for at least 80% of CLL patients, including all CLL patients with poor prognosis as assessed by the presence of the deletion 11q (ATM) or the deletion 13q1q (p(53)). Furthermore, it allows easy standardization among laboratories, applying FACS cell sorting, as it is based on a two-colour labelling only and on FISH assays using commercially available probes. We now clinically validating the method by assessing MRD levels in intensively treated CLL patients and we propose this method as a candidate approach for assessing the clinical impact of MRD detection in prospective clinical trials on CLL.

**0275**

**MRD KINETIC AFTER AUTOLOGOUS STEM CELL TRANPLANTATION IN CHRONIC LYMPHOCYTIC LEUKAEMIA CAN PREDICT INDIVIDUAL TIME TO RELAPSE AND IS ASSOCIATED WITH CLINICAL OUTCOME AND IGVH MUTATIONAL STATUS**

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**Introduction.** Minimal residual disease (MRD) short after autologous SCT in pts with CLL is known to be close to the detection limit in patients in hematologic remission. Therefore early MRD after SCT is not a predictor of outcome in patients in remission after SCT. Nevertheless, outcome after SCT is rather heterogeneous even in this population and it has been shown, that the IGH mutational status and other risk factors are of prognostic value after SCT. We therefore aimed to study MRD in 61 patients with high risk CLL after autologous conditioning regimen of TBI and high-dose cyclophosphamide and consecutive autologous SCT. We established a mathematical model to describe the individual kinetics of MRD-increase after SCT and correlated this to known risk factors as IGH mutational status, cytogenetics, Lymphocyte doubling time, STK, leukocyte count and clinical outcome. We therefore plotted LOG-MRD levels in each individual patient against time after SCT for an observation period between 12 and 36 months after SCT and calculated patient individual standard curves by linear regression. Significant MRD increase was defined by a change of more than 0.5 orders of magnitude within this observation time, all other cases were regarded as MRD stable or decreasing. 31 of 61 patients showed increasing MRD level with a median slope of 0.08 (0.04-0.88). Assuming that MRD level of 0.5 would be diagnosed as hematologic relapse we could predict the individual clinical relapse by extrapolation with high accuracy (median difference between predicted and observed relapse 1.6 months) in 29 relapsed patients. Patients in ongoing remission showed a significant smaller slope and longer time to predicted relapse compared to relapsed patients (0.09 vs. 0.05 and 48.1 vs. 62.5 months respectively). More important the slope of patients with unmutated VH genes which significant steeper than in mutated cases (0.09 vs. 0.01; p=0.004) whereas other risk factors as LT, leucocyte count, 11q deletions, or CD23 (ATM) did not show significant difference in MRD kinetics. None of these parameters had significant influence on the MRD level within the first year after SCT. Conclusions. LOG-linear MRD models can characterize CLL increase after SCT:

Increasing MRD kinetic predicts the time-point of the clinical relapse with acceptable accuracy in the majority of CLL pts post SCT, whereas decreasing or stable MRD levels are associated with long lasting remission. Absolute MRD levels after SCT are homogeneous low regardless known risk factors, but fast increase of the relapsing CLL clone is correlated to VH unmutated cases. This indicates that the dismal outcome of VH unmutated cases is based on higher proliferating capacity compared to VH mutated cases and not on chemoresistance.

**0276**

**MDR-1, BUT NOT MDR-3 GENE EXPRESSION, IS ASSOCIATED WITH UNMUTATED IGH GENES AND POOR PROGNOSIS CHROMOSOMAL ABERRATIONS IN CHRONIC LYMPHOCYTIC LEUKAEMIA**

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**Background.** The intrinsic or acquired resistance to anticancer drugs remains one of the most significant factors impeding progress in cancer chemotherapy. Although the cellular basis underlying multidrug resistance (MDR) is not fully understood, several factors mediating therapy resistance in tumours have been proposed. One of the mechanisms leading to chemotherapy resistance in tumour cells is the increased activity by members of the ATP-binding cassette (ABC) superfamilly of transport proteins, which function as energy-dependent drug efflux pumps. Two multidrug resistance genes have been identified in humans, MDR-1 and MDR-5. The clinical and biological significance of these MDR mechanisms has not been sufficiently investigated. We therefore studied whether expression of these MDR genes was dependent on prior exposure to therapeutic agents. Methods The presence of MDR-1 and MDR-3 was determined using the alkaline phosphatase anti-alkaline phosphatase (APAAP) technique. IGHV mutational status, gene usage, CD83 data, FISH analysis and clinical data were available on all patients. Results. One-hundred and one, and 25 patients were tested for the presence of MDR-1 and MDR-1 expression, respectively. Positive and negative controls were included in each batch of APAAP. Twenty-one of 101 patients showed MDR-1 positivity, though no significant associations between MDR-1 gene expression and markers of poor prognosis or exposure to therapeutic agents were evident. MDR-3 expression (19/25) showed a strong association with unmutated IGHV genes and adverse cytogenetics (p=0.015, p=0.014, respectively). Four patients showed co-expression of MDR-1 and MDR-3, 2 of whom have succumbed to their disease. Conclusions. In keeping with previous reports, this study demonstrated that expression of both MDR genes is independent of prior exposure to therapeutic agents. Additionally, no associations between advanced clinical stage and expression of MDR genes were evident. MDR expression was, however, associated with shorter survivals than in MDR negative patients. This study highlights the value of determining MDR phenotype in CLL patients, in both refractory and untreated patients. This would allow the design of novel drug regimens containing agents that reverse MDR function, in combination with conventional therapeutic drugs, with the prospect of improving outcomes in CLL.

**0277**

**ANTILEUKEMIC ACTIVITY OF LENALIDOMIDE (REVLIMID) IN PATIENTS WITH RELAPSED OR REFRACTORY CHRONIC LYMPHOCYTIC LEUKAEMIA**

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**Introduction.** The ImiDs are a new class of immunomodulating agents with antitumor activity against various malignancies. We previously reported the antileukemic activity of thalidomide (T) in combination with budesonide in CLL pts. Based on this experience we investigated the more potent analog of T in pts with rel/ref CLL. Here we report the results of this ongoing phase II clinical trial. Patients and Methods. All pts...
with rel/ref CLL requiring treatment are eligible. Oral L is given at 25mg/day for 21 out of a 28-day cycle. Treatment is continued until complete response (CR) or progressive disease (PD). NCI-WG 1996 criterion is used to determine response. Three pts had PD and rituximab was added to L. All patients have achieved a PR. (reported separately).

**Results.** Nineteen of the 29 pts (median age 64 years; range: 47-75) enrolled are evaluable for response. Duration of monotherapy ranges from 7 to 16 months and combination therapy with Rituximab ranges from 1 to 6 months. Ten pts are ineligible (3 withdrew consent and 7 received <2 months of therapy due to toxicity). Major response was noted in 16 of 19 evaluable pts (84%) with CR (2 molecular remission) and 15 PR. All CR’s have received monotherapy. **Toxicity:** Most common grade 3/4 adverse effects were tumor lysis (60%) and neutropenia (55%). Another common AE was tumor flare; characterized by tender swelling of lymph nodes and/or rash and low-grade fever, noted in >80% of the pts. One patient developed a DVT and one incurred a PE.

**Conclusion.** L is clinically active in CLL pts with rel/ref disease. Hematologic toxicity was the most common AE requiring dose reduction. Overall SE profile was predictable and manageable.

**0278**

**ZAP-70 EXPRESSION IN B-CLL IS ASSOCIATED WITH INCREASED RISK OF AUTOIMMUNE CYTOPENIAS**

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**Background.** Autoimmune cytopenias (AIC), namely autoimmune hemolytic anemia (AIHA), thrombocytopenia (AITP), Evans Syndrome (ES) and pure red cell aplasia (PRCA), are relatively common complications of B-CLL. The risk of AIC is higher in advanced and heavily treated B-CLL patients. The prognostic impact of AIC on survival is still questioned. **Aim of the study.** To assess the possible correlation of ZAP-70 expression, which is a well-documented prognostic factor in B-CLL, with AIC occurrence. Methods. We retrospectively evaluated the incidence of AIHA, AITP, ES and PRCA in 233 B-CLL patients tested for ZAP-70 expression in leukemic cells by immunohistochemistry on bone marrow biopsies (184) and/or flow cytometry on peripheral blood performed within 6 months from diagnosis. Results. They were 136 males (58%) and 97 females, aged 34 to 84 years (median 65). At presentation 184 (79%) were Binet stage A, 38 (16.3%) stage B and 11 (4.7%) stage C. Median follow-up was 62 months (range 12 to 387). Overall, AIC was observed in 23 patients (9.8%), 15 were AIHA, 2 AITP, 2 ES and 1 PRCA. In 7 cases (30%) the complication was present at diagnosis (3 AIHA, 2 AITP, 1 ES, 1 PRCA), in the remaining it appeared subsequently, mostly after treatment (alkylating agents in 13, fludarabine in 2). ZAP-70 was expressed in leukemic cells of 18/23 (78%) patients with AIC. The actuarial cumulative incidence of AIC at 10 years was 53±10% in ZAP-70 positive vs 7±5% in ZAP-70 negative cases (p=0.0004). In B-CLL patients developing AIC, survival was lower (24±14% vs 80±4% at 10 years, p=0.0003). Overall survival of all ZAP-70 positive B-CLL patients was significantly shorter (44±9% at 10 years vs 88±4% of ZAP-70 negative cases p=0.00004). ZAP-70 expression was the only significant factor for developing AIC at multivariate analysis (p<0.02). No significant association with age, sex, Binet stage, lymphocyte count or previous B-CLL treatment was found. **Conclusions.** Our data suggest that ZAP-70 expression in leukemic cells is independently strongly associated with the risk of autoimmune cytopenias in B-CLL. A possible pathogenetic suggestion might be related to the enhanced signalling via BCR complex induced by ZAP-70.

**0279**

**IN VITRO TREATMENT OF CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) CELLS WITH FLUDARABINE, ETOPOSIDE AND ALEMTUZUMAB (CAMPATH-1H) LEADS TO SPECIFIC MECHANISMS AND RATES OF CELL DEATH IN DIFFERENT GENETIC SUBGROUPS**

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**Background.** Treatment of CLL with fludarabine, etoposide and the monoclonal anti-CD52 antibody alemtuzumab leads to cell death and clinical responses. The mechanisms by which these processes occur are poorly understood. **Aims and Methods.** In order to gain insight into these mechanisms CLL cells from 41 patients were collected and individually treated with fludarabine (500 µM), etoposide (60 µM) and alemtuzumab (10 mg/ml cross-linking f(ab')2 fragments) for 24 and 48 hours respectively. Each sample treated with alemtuzumab was also cultured with and without allogeneic serum as a source of complement. In 21 cases T-cell and NK-cell depletion was done using negative selection with anti-CD2 and anti-CD14 magnetic beads. Of 38 cases investigated 25 were VH unmutated and 12 of 39 had del 11q and/or del 17p (n=3). FACS analysis was used to measure rates of cell death with double staining for Annexin V/7AAD and caspase-3 activation. **Results.** Treatment with fludarabine and etoposide induced apoptosis in all but 2 cases, which carried del 17p and del 11q respectively. The rates of apoptosis were lower in cases with genetically high-risk (del 11q or del 17p) CLL, although these cells showed stronger caspase-3 activation than low-risk CLL cells when incubated with fludarabine (see table). Response to alemtuzumab was highly dependent on the presence of serum in the culture. 7% Annexin-V/7AAD-positive cells in serum-free cultures vs 67% in cultures with serum. Addition of f(ab')2 fragments increased the percentage of Annexin-V/7AAD-positive cells even in serum-free cultures. Response to alemtuzumab was independent of the genetic subgroup of the case. Notably, treatment with alemtuzumab in serum containing cultures did not produce cells that stained Annexin-positive/7AAD-negative, a typical feature of early apoptosis, whereas treatment with Fludarabine, etoposide and alemtuzumab in serum-free medium resulted in a significant number of Annexin-positive/7AAD-negative cells. This was also observed in T-cell-depleted cultures. In the presence of serum, alemtuzumab did not induce caspase-3 activation, neither did the addition of f(ab')2 fragments. However, in serum-free cell cultures, active caspase-3 was clearly detectable after alemtuzumab treatment, and caspase-3 activity was further up-regulated when f(ab')2 fragments were also added. **Summary.** After in vitro treatment of CLL cells with fludarabine, etoposide and alemtuzumab mechanism and rate of cell death differed significantly depending on the genetic subgroup affiliation. CLL cells with high-risk aberrations were more capable of caspase-3 activation when treated with fludarabine or alemtuzumab. Alemtuzumab killed CLL cells independently of serum as a source of complement, but the mechanism of response was different and more effective when serum was added. In serum-free CLL cultures, alemtuzumab induced apoptosis with activation of caspase-3, and addition of cross-linking f(ab')2 fragments increased the rate of apoptosis, whereas in the presence of serum treatment with alemtuzumab induced no typical features of apoptosis, even in T-cell depleted cultures. These findings favor a combination of both CDC and apoptosis but not of ADCC, as the cell kill mechanisms activated by in vivo alemtuzumab.

**Table 1.**

<table>
<thead>
<tr>
<th>Mean % of cells Annexin V+/7AAD+</th>
<th>Caspase-3 activation</th>
<th>end</th>
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<tr>
<td>Mean caspase-3 activity (%)</td>
<td>(etoposide 48 hrs)</td>
<td>fludarabine (48 hrs)</td>
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<td>--------------------------------</td>
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</tr>
<tr>
<td>7% Annexin-V/7AAD-positive cells</td>
<td>33%</td>
<td>29%</td>
</tr>
<tr>
<td>7% Annexin-V/7AAD-positive cells</td>
<td>58%</td>
<td>35%</td>
</tr>
<tr>
<td>del 11q/del 17p</td>
<td>15%</td>
<td>25%</td>
</tr>
<tr>
<td>13q/g normal karyotype</td>
<td>11%</td>
<td>32%</td>
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ANALYSIS OF EXPRESSED AND NON-EXPRESSED IMMUNOGLOBULIN LAMBDA LOCUS REARRANGEMENTS IN CHRONIC LYMPHOCYTIC LEUKEMIA

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In normal individuals, nearly all lambda expressing B cells have rearranged immunoglobulin kappa (IGK) genes and carry IGKV-J junctions, whereas only 2-3% of kappa expressing cells carry IGLV-J junctions. We have recently reported IGK locus rearrangements in the vast majority (97%) of lambda-CLL cases. In the present study, IGL locus rearrangements were analyzed in parallel on cDNA/genomic DNA in 165 kappa- and 104 lambda-CLL cases. In all cases, the tumor load was greater than 70%. All experiments were repeated at least three times with identical Results. Furthermore, in 156/267 cases repeat samples (obtained at different times) were analyzed and gave identical Results. In lambda-CLL, 110 IGLV-J transcripts were amplified in 104 cases. Two cases carried double in-frame (IF) transcripts: in such cases, the possibility that leukemic cells expressed more than one lambda chain cannot be excluded. Four out of 110 IGLV-J transcripts were out-of-frame (OF); 2/4 OF transcripts were heavily mutated and carried stop codons. DNA-PCR identified additional, non-transcribed IGLV-J rearrangements in 6/104 lambda-CLL cases, of which only one was in-frame. The most frequent genes in transcribed, in-frame rearrangements were IGLV2-21/IGLV3-1/IGLV3-14/IGLV3-1/IGLV1-40. LCDR3 median length was 11 amino acids (range, 8-13); N nucleotides were detected in 50/106 (47.2%) IGLV-J joints; 84/106 cases (79.2%) used the IGLJ2/3 genes, whereas the remainder (22/106, 20.8%) used the IGLJ1 gene. Non-transcribed and out-of-frame rearrangements utilized 9 different IGLV genes and had a median LCDR3 length of 11 amino acids (range, 10-13). N nucleotides were detected in 57/106 IGLV-J joints; 8/10 cases (80%) used the IGLJ2/3 genes. In kappa-CLL, IGLV-J rearrangements were amplified in 10/165 patients (6.1%); 6/10 rearrangements were in-frame. Eight different IGLV genes were identified. Somatic mutations were introduced in 8/10 IGLV sequences. Four out of ten IGLV-J rearrangements in kappa-CLL were also transcribed; 3/4 IGLV-J transcripts were in-frame. In the three kappa-CLL cases with transcribed, in-frame IGLV-J rearrangements, flow cytometry and immunohistochemistry demonstrated that monocytic IgE expression was still maintained. In particular, malignant B cells were negative for either cytoplasmic or surface lambda light chains, suggesting post-transcriptional regulation of allelic exclusion. IGLV-J rearrangements in kappa-CLL had a median LCDR3 length of 10 amino acids (range, 9-12); N nucleotides were detected in 5/10 IGLV-J joints; 4/10 cases (40%) used the IGLJ2/3 genes, whereas 6/10 cases (60%) used the IGLJ1 gene. In conclusion, biallelic IGL locus rearrangements are infrequently detected in lambda-CLL. A small subset of lambda-CLL patients have cells that may express more than one lambda chain allele, implying that allelic exclusion of light chains is not absolute. IGL locus rearrangements are infrequent in kappa-CLL, suggesting that the light chain rearrangement hierarchy in chronic lymphocytic leukemia (CLL) is not inherently different from normal cells. Differences in IGLJ gene usage between kappa vs. lambda-CLL can indicate negative selection of the IGLJ gene in the expressed CLL repertoire.
Background and objective. In the HAART era, the results of therapy of HIV-related lymphomas are similar to those observed in non-immunocompromised patients. There is scarce information on the results of therapy of HIV-related HL using standard chemotherapy together with HAART. The aim of this study was to analyze the results of ABVD regimen + HAART in a multicenter series of 62 Spanish patients with HIV-related HL in advanced stages. Patients and Methods. From 1996 to 2005, 62 HIV-infected pts with newly diagnosed HL were treated in 15 Spanish hospitals. HAART was given to all patients from diagnosis if they were not receiving it already. Six to eight cycles of ABVD were planned. G-CSF support was administered according to institutional practices. Response to chemotherapy as well as prognostic factors for response, OS and DFS were recorded. Results. Median age 57 yr (range 24-61), 54 (87%) males, 29 (47%) with previous known diagnosis of HIV infection (median from HIV infection diagnosis to HL: 5 yr, range 0-10). Risk activity for HIV infection: IV drug abusers 33 (53%), heterosexual 15 (24%), homosexual / bisexal 13 (21%), unknown 1 (2%). Median CD4 lymphocyte count <100/ml, 22 (35%), median HIV load: 1.4×10^3 copies/ml (range 0.3-9×10^5), undetectable HIV load: 21/56 (37%). Forty seven (76%) patients were receiving HAART at the time of HL diagnosis (median 15 mo, range 1-109). HL subtype: nodular sclerotic 17 (27%), mixed cellularity 25 (41%), lymphocyte depletion 10 (16%), non-specified HL 10 (16%). The main reason was diagnosis in extranodal areas. ECOC score ≥2, 22/53 (42%), B symptoms: 55 (89%), stage III: 21 (34%), stage IV: 41 (66%), BM involvement: 33/60 (55%). Treatment with the scheduled 6-8 ABVD cycles was completed in 81% of cases. Induction death: 5 pts (8%), CR: 54 (87%), resistance 3 (5%). After a median follow-up of 44 mo, 5 pts have relapsed, with a DFS probability at 5 yr of 74% (95% CI 46-100), and 15 patients have died, being 5-yr OS probability 76% (95% CI 23-89). Causes of death: lymphoma progression 10, HIV-related 3, traffic accident 1, unknown 1. Virologic response to HAART at 6 months after the completion of treatment was observed in 24/25 (67%) evaluable patients. Only lower number of ABVD cycles than scheduled (≤6) was a prognostic factor for CR achievement. DFS and OS (OR: 0.153, 95% CI: 0.051-0.462, p=0.001 for CR, OR: 0.137, 95% CI: 0.019-0.979, p=0.05 for DFS, and OR: 0.153, 95% CI: 0.051-0.462, p=0.001 for OS). Conclusion. Patients in advanced stage, HIV-related HL treated with ABVD+HAART have a response rate and survival similar to that of immunocompetent patients. The completion of the scheduled chemotherapy was the only factor influencing response and survival in this series.

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Hodgkin’s Lymphoma in Adolescents - Results from the German Hodgkin Study Group

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Background. Both pediatric and adult patients with Hodgkin lymphoma (HL) are commonly treated in separate treatment protocols. Adolescents are treated with either pediatric or adult protocols depending on study group policies and local legislation. Between 1988 and 1998, the German Hodgkin Study Group (GHSG) did include younger patients from age of 15, and 16, in identically therapy regimens with adult Hodgkin’s lymphoma patients. Aims. With a focus on treatment outcome and the recording of secondary malignancies, this analysis aimed to demonstrate whether adolescent patients with HL represent a patient group distinct from adults, possibly requiring a separated therapy strategy. Methods. In two GHSG trial generations (G2, and G3), a total of 573 adolescents (15-21 years) in early, intermediate, and advanced stages HL were compared with 4544 adults (22-65 years) for complete remission rate (CR), 5 years survival rate (SV), 5 years freedom from treatment failure (FFTF), and secondary neoplasias (2nd NHL). Results. For both ado-lescents and adults, treatment outcome showed no differences in all stages in terms of CR, SV, and FFTF. A higher rate of 2nd NPLs in the adults patient cohort was detected consistently for early, intermediate, and advanced stages in both trial generations. However, the absolute number of 2nd NPLs in the adolescent group was generally low. Conclusion. Adolescent and adult patients suffering from Hodgkin’s lymphoma show similar therapy outcome when treated with the same regimens. With respect to the small number of cases, a longer follow up is needed to assess the risk of 2nd NPLs particularly in adolescents. Based on this analysis, adolescents should not be a distinct patient group with the need for a treatment strategy apart from adult Hodgkin’s lymphoma patients.
Background. Despite a high cure rate, 10 to 40% of patients with HL may relapse after complete remission and 5% are refractory to ABVD. Many regimens have been used as salvage chemotherapy with response rate ranging from 40 to 50% depending on patients status (relapsing or refractory). Since 1998, MOPP/ABV chemotherapy has been abandoned and most patients are treated with first line ABVD with cumulative dose of doxorubicin often over 300 mg/m². Aims. For these reasons we developed a second line treatment without doxorubicin including ifosfamide/ etoposide and eloxatin (a platinum component without nephrotoxicity) to reduce disease before high-dose therapy (HDT) and ASCT. Methods. the IVOX (ifosfamide 1500 mg/m² etoposide 150 mg/m²/D1D2D3 and eloxatin 130 mg/m²/D1) was given every 21 days with GCSF day 6 for 6 days. Twenty one patients with progressive HL (median age 29y) have been prospectively treated from 06/05 to 06/06. Characteristics of patients: initial stage III/IV (n=11) bulky mediastinum (n = 8), all patients had received ABVD or epirubicin in 5 cases (EBVP) with radiotherapy in 10 cases. At progression 5 patients were induction failure and 16 were in unfavourable relapse (mean time to relapse at 8 mo., stage III/IV at relapse 60%). Patients were evaluated after 2 or 3 IVOX with a PET CT and had PBFC collection before HDT. Results. according to standard staging criteria 7 patients were in CR/CBr and 7 in PR >50% giving a response rate of 66.6% and according to PET evaluation, 10 patients had a positive PET CT before intensive therapy. The toxicity was low without hospitalisation for febrile neutropenia, no transfusion, no mucositis. 19 patients had a successful PBFC (2 patients were excluded from PBFC collection, one 66 y with refractory disease and one for viral hepatitis). Among the 19 patients planned for HDT, one died with refractory disease and 18 received HDT (Tandem in 9 cases andRIC allogeneic in one case). At the last follow-up 16 patients are in CCR (with negative PET CT), 2 died from HL and 3 are alive with disease. Conclusion IVOX is a very well tolerated chemotherapy regimen but doesn’t appear superior to previous published regimens in progressive HL.


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Background. Lymphocyte-predominant Hodgkin’s lymphoma (LPHL) is characterized by early stage, indolent course, excellent prognosis but high risk of second tumors in part treatment-related. In order to clarify the treatment strategy, SFCE has reported its initial experience with a wait and see strategy after adenectomy in a limited number of patients (J Clin Oncol Pellegrino et al., 2003). Aims: to further document patients LPHL s evolution when they received no treatment beyond initial adenectomy. Methods. From 1990 to December 2005, 59 patients with LPHL confirmed after pathological review were available for the study. Clinical presentation was: 47 male; median age 10 years (4-17); stage I n=45, stage II n=8, stage III n=5, stage IV n=1. Based on physician decision, 22/45 stage I patients received no further treatment after initial surgery (group S). 23 patients (group CT) received: combined treatment (n=10), involved field radiotherapy alone (n=3) or chemotherapy alone (n=10). None of them received monoclonal anti-CD20 antibody. The 2 groups were comparable for clinical status and follow up. Results. 45/48 achieved CR. All patients with residual lymph node relapsed. With a median follow up of 41 months (6-156), overall survival is 100%. Overall DFS stage I patients is 57% ± 10, DFS group SA: 52% ± 14 and DFS group CT: 65% ± 13 (p = 0.2). Only two patients had TEP-FDG for post surgical evaluation. Median relapse time is 11 months (SA group 5 months/CT group 25 months p = 0.2). Stage at relapse was SA group: 5/7 in the same node area and 2/7 stage II; CT group: 4/6 stage I, 1/6 stage III and 1/6 stage IV. Conclusions: No further therapy after complete lymph node resection is a valid approach in LPHL comparable to more aggressive approaches. Nevertheless, as most of the relapses involve the same site than the diagnostic a better evaluation of the quality of remission after surgery is to be recommended with TEP and CT/MRI. This could help for an adapted therapeutic approach.
Background. Socioeconomic status (SES) is a determinant of clinical outcome of Hodgkin’s disease (HD). Aims. To analyse the impact of the socioeconomic status in patients with Hodgkin’s disease (HD). Methods. From November 2001 to January 2005, 194 consecutive patients were prospectively followed in five institutions (three public and two private) in Rio de Janeiro. Data regarding disease and treatment features were collected, and patients were classified according to the International Prognostic Score (IPS). Each patient answered a questionnaire about their socioeconomic status, including educational level, household income, ownership of household goods (radio, TV, refrigerator, washing machine, VCR/DVD and car), presence of housemaid, and housing features. Most of these items were used to calculate an index of socioeconomic status, the ‘Criteria for Economic Classification’. The index has been validated in public and private institutions in Brazil. Patients were divided in two groups according to their socioeconomic status: higher SES (classes A1 to C) and lower SES (classes D and E). The IPS score risk was also categorized in low risk (2 or less risk factors) or high risk (more than 2 risk factors). Results. There were 151 patients (78%) with a higher SES and 43 patients (22%) with a lower SES. The overall CR rate was 82%, and it was higher in patients with a low risk IPS (87% vs. 72%, p=0.04). Patients with a higher SES had a higher CR rate than those with a lower SES (85% versus 72%, p=0.066, 95% CI of difference:0.45% to 26.22%). The median albumin level at diagnosis was lower in the lower SES group (3.55 versus 3.9, p=0.067) and median CR rate was higher in the lower SES group (87% versus 72%, p=0.018). There were no statistically significant associations between the SES group and other relevant variables, including stage, bulky disease performance status, and time from the beginning of symptoms to diagnosis. Ten patients (5%) died during treatment. The causes of death were related to the disease, including advanced disease in 3, and cachexia in one patient. Death during treatment was associated with a lower SES (16% vs. 2%, p=0.001), a lower performance status (p=0.001), a lower lymphocyte count (p=0.012), and weakly with a lower albumin level (p=0.065). With a median follow-up of 1.7 years (0.07–4.35), a higher SES was associated with a better 2-year overall survival (87% versus 79%, p=0.01). Summary/Conclusion. Lower socioeconomic status was associated with an increased rate of fatal events during treatment, and with a trend towards a lower complete remission rate. Overall survival was lower in the socially deprived patients, apparently due to the higher fatality rate during treatment. Factors indicative of a poor health status at the time of diagnosis appear to explain the observed differences in outcome. In underprivileged countries, patients with a lower socioeconomic status require a more careful monitoring during treatment, possibly with specific support measures. Regimens more intensive than ABVD could pose a prohibitive risk of complications in this group of patients.

0289 POLYMORPHISMS OF GLUTATHIONE S-TRANSFERASE MU1 AND TETA 1 GENES IN HODGKIN’S LYMPHOMA RISK

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Background. Hodgkin’s lymphoma (HL) is a heterogeneous malignancy, and little is known about the aetiology of this disease. The environmental exposure to cytotoxic and genotoxic agents may be associated with an increased risk of HL. The ability to metabolise carcinogens is variable in human beings. The enzymes of the glutathione S-transferase (GST) system catalyse the conjugation of electrophilic molecules of numerous carcinogenic chemicals, such as benzene and polycyclic aromatic hydrocarbons, to glutathione reducing them to less toxic levels. Genes coding for GST mu1 (GSTM1) and theta1 (GSTT1) proteins are polymorphic in humans and are absent or homozogous null in 10–60 percent of different ethnic populations. Differences in carcinogen metabolism may explain differences in cancer susceptibility. The association of the GST null genotype and the risk of developing HL are not yet fully clarified. Aims. We have tested whether null genotypes for GSTM1 and GSTT1 genes altered the risk for the disease in Brazilian patients. Method. For this purpose, genomic DNA from peripheral blood of 79 HL patients (40 male, 39 female; mean age±SD: 32.2±14.9 years) and peripheral blood of 367 controls (196 male, 169 female; mean age±SD: 53±4.6 years) was extracted using proteinase K and lithium chloride protocol. GSTM1 and GSTT1 gene amplification was performed by polymerase chain reaction (multiplex PCR method), including amplification of the globin gene fragment used as a control of the DNA sample. Statistical significance of the differences between groups was calculated by chi-square or Fischer exact test. Crude odds ratios (ORs) were calculated and were given within 95% confidence intervals (CI). Results. We have identified a lower prevalence of GSTM1 (49.4%) and GSTT1 (17.7%) null genotypes in HL patients and controls (42.5% and 18.0%, respectively; p=0.062 and p=1.00). No significant difference was also found in the GSTM1 and GSTT1 null genotype frequencies in either group (7.9% vs 11.4%; p=0.35). Our observation of a 1.32-fold (95%CI: 0.81–2.15) and 0.98-fold (95%CI: 0.52–1.86) risk associated with GSTM1 (null genotype: 16% vs. 2%) and a 1.54-fold (95%CI: 0.67–3.54) risk associated with the combined null genotype. Conclusions. These results suggest that the inherited absence of this carcinogen detoxification pathway may be unimportant determinant for the HL, but a larger number of patients from distinct populations should be analysed to clarify this issue.

Supported by FAPESP

0290 LACE (LOMUSTINE, ARA-C, CYCLOPHOSPHAMIDE, ETOPOSIDE) CONDITIONED AUTOLOGOUS STEM CELL TRANSPLANTATION FOR RELAPSED OR REFRACTORY HODGKIN LYMPHOMA: TREATMENT OUTCOME AND RISK FACTOR ANALYSIS IN 67 PATIENTS FROM A SINGLE CENTRE

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Background. High-dose chemotherapy followed by autologous stem cell transplantation (ASCT) is a recognised treatment option for patients (pts) with relapsed Hodgkin lymphoma. Patients and Methods. We analysed 67 pts (46m, 21f, median age at diagnosis 29y, range15-67) who underwent autologous stem cell transplantation (ASCT) after LACE [lomustine (CCNU), cytarabine (Ara-C), cyclophosphamide, etoposide] conditioning for relapsed (n=61) or primary refractory (n=6) Hodgkin lymphoma. The predominant diagnostic histology was nodular sclerosis (n=42), whilst disease stage was I in 2pts, II in 29pts, III in 22pts and IV in 14pts. Median age at ASCT was 52 y (range: 17-70). Prior to ASCT, 40 pts were in complete or partial remission, but 27 pts had less than partial remission. Results. The 100 day treatment-related mortality was 3%. With a median follow-up of 43.5 months (range 0.8–145.5) the probabilities of overall survival (OS) and progression-free survival (PFS) at 5 years for all 67 patients were 72% and 61%, respectively. Probabilities for OS and PFS at 5 years for patients with chemo-sensitive relapse (n=40) were 79% and 76%, versus 42% and 39% respectively for patients (n=27) with chemo-resistant relapse (p=0.056 for OS, p=0.005 for PFS). In univariate analysis gender, age at diagnosis or at ASCT, extranodal disease, bulk or bone marrow involvement at diagnosis, initial treatment type, response to first line chemotherapy, duration of first remission, time from diagnosis to ASCT, number of treatment lines before ASCT did not have an impact on OS. The following risk factors for worse OS were identified in multivariate analysis (mixed cellularity or lymphocyte-depleted histology, stage III or IV disease at diagnosis, and haemoglobin ≤10g/dl at ASCT). Patients with 0 (n=12), 1 (n=26), or 2-3 (n=17) of these three risk factors had 5-year OS probabilities of 100%, 75% and 32% respectively. Conclusions. We conclude that LACE followed by ASCT is an effective treatment for the majority of patients with chemo-sensitive relapsed Hodgkin lymphoma. A proportion of apparently chemo-refractory patients may also benefit.

0291 67GA-SPET HAS A ROLE IN PREDICTING DISEASE FREE SURVIVAL (DFS) AND OVERALL SURVIVAL (OS) IN PATIENTS WITH PRIMARY MEDIASTINAL LYMPHOMA AND HODGKIN LYMPHOMA WITH MEDIASTINAL INVOLVEMENT

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Background. FDG-PET has a superior accuracy than gallium scan (Ga-SPET) in staging and post-therapy restaging of malignant lymphomas. However, in Hodgkin lymphoma (HL) with predominant mediastinal involvement and in primary mediastinal lymphoma (ML), this latter, less expensive, nuclear imaging technique might still have a clinical utility. Aims. The main objective of this prospective study was to assess the predictive value of Ga-SPET in terms of DFS and OS. Methods. Ga-SPET was performed 72 hours after intravenous injection of 370 MBq (8-10

11th Congress of the European Hematology Association
Results: The actual final analysis includes 66 evaluable patients (mean age 28, range 12-80) of the 68 initially enrolled in this prospective study. The main disease features of the 66 (43/23/FA) patients are: stage II/III, III/IV, 1I/11V; histology HL/SN/42, CM/11, DL/1, LP/1, unclassified/5) and ML/8; bulky mediastinal disease yes/no 29/37, B symptoms yes/no, 41/25. Forty-two patients received conventional chemotherapy, 4 non-mycobacterial and 20 myeloablative chemotherapy with peripheral blood stem cell support because of unfavourable disease or resistant or relapsing disease to primary treatment. Two patients were excluded because they did not have Ga-SPET at the end of treatment. A total of 109 Ga-SPET/CT restaging were obtained after chemotherapy and/or chemo-radiotherapy completion. Sensitivity, specificity and accuracy were 89%, 91% and 91%, respectively for the Ga-SPET, while they were 100%, 27% and 37% for the CT scan. After a median follow-up of 34 (4-141) months, DFS and OS for patients with a pathological Ga-uptake (Ga-SPET) at the end of the treatment program were 95 (2-60) and 26 (9-70) months, respectively. In contrast, the corresponding figures in patients with pathological Ga-uptake after treatment as compared with patients with a negative Ga-SPET. In contrast, DFS and OS were not significantly different between patients with post-treatment CT scans suggestive of persistent disease and patients with CT scans indicating a complete disease remission (Figure). Conclusions. Ga-SPET is still a useful, sensitive and not expensive method to determine the presence of eventual post-therapy active disease in the mediastinum.

0292
AUTOLOGOUS STEM CELL TRANSPLANTATION FOR PATIENTS WITH REFRACTORY OR RELAPSED HODGKIN LYMPHOMA: CLINICAL OUTCOME OF 61 PATIENTS FROM A SINGLE INSTITUTION

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Background. Patients with Hodgkin lymphoma (HL) who do not achieve complete remission (CR) with conventional chemotherapy have poor prognosis. The treatment of these patients is high-dose chemotherapy and autologous stem cell transplantation (ASCT) that may result in prolonged progression-free survival as shown in many studies, particularly registry-based. Aim. To investigate the results of ASCT in patients from a single institution with refractory or relapsed HL at the time of the procedure. Patients and Methods. Sixty-one patients, 27 males and 34 females with a median age of 31 years (range 15-60) transplanted from 1988 to 2005 were analysed. All patients had active HL at the time of ASCT: 51 patients were in partial remission (50%), 18 had refractory disease (30%), and 12 had a non-treated relapse (20%). At transplantation, 26 patients (48%) had advance stage, 15 (25%) present-
Hodgkin Lymphoma - Clinical trials

0294
CLINICAL RESISTANCE TO PRETRANSPLANT IMATINIB THERAPY IS AN ADVERSE PROGNOSTIC FACTOR FOR THE OUTCOME OF ALLOGENIC STEM CELL TRANSPLANTATION IN CML

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ment of full donor CD34+ T cell chimerism (TCC) in a series of 102 patients receiving RIC allo-SCT from an HLA-identical sibling. 65 patients (64%) received an ATG-based RIC regimen (fludarabine, busul- fan and ATG), 14 patients (14%) received a low dose TBI-based RIC (2 Gy total dose), while the remaining 23 patients (23%) received an association of fludarabine, busulfan and total lymphoid irradiation (TLI). At day 30, 77% (95% CI, 69-85%) of patients had a full donor TCC. In univariate analysis, none of the patients’, graft, RIC type, or disease characteristics were predictive of establishment of an ‘early’ full donor TCC at day 30 after allo-SCT. However, the group of 51 patients who achieved a full donor TCC by day 30, had a significantly higher incidence of grade 2-4 acute GVHD, in comparison to the group of 71 patients who were still in mixed TCC at day 30 (cumulative incidence, 61% vs. 35%; p=0.01). When looking for predictive factors for full donor TCC at day 90, univariate analysis showed that diagnosis category, the RIC regimen type (ATG, TBI or TLI-based RIC), a female donor, CD34+ stem cell dose, and CD4+ T cell dose infused, were significant or had a trend towards significant association with the establishment of full donor TCC by day 90. In the multivariate analysis, a diagnosis other than myeloid malignancy, was the strongest parameter significantly predictive of establishment of full TCC at day 90 after RIC-allo-SCT (p=0.007; OR=3.82, 95%CI, 1.4-10.1). Most importantly, the delayed establishment of full donor TCC in patients with myeloid malignancies translated towards a worsened PFS (p=0.06) in the group of 15 patients who did not achieve full donor TCC at day 90 as compared to the group of 26 patients who achieved a full donor TCC. This worsened PFS was due to a significantly higher incidence of leukemia relapse among these 15 patients (40% vs. 20%) as compared to none in the other group of 26 patients (p=0.002). We conclude that cautious monitoring of the levels of donor TCC is mandatory after RIC allo-SCT, because this can improve patients’ outcome through identification of patients at risk for acute GVHD, and disease progression, and guidance of early interventions with immunosuppressive drugs or donor lymphocyte infusions aimed at obviating these complications.

**0299**

**ALLOGENEIC HEMOPOIETIC STEM CELL TRANSPLANTS (HSCT) FOR PATIENTS WITH RELAPSED ACUTE Leukemia : LONG TERM OUTCOME**


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**Background.** Patients with acute leukemia may be referred for allo-geneic hemopoietic stem cell transplantation (HSCT) at the time of relapse. The outcome of allogeneic HSCT in these patients is relevant when discussing treatment strategies and donor selection. Aim of the study. To assess the long term outcome of 152 patients with acute myeloid (AML) or acute lymphoid leukemia (ALL) undergoing an allogeneic HSCT in our Unit between 1977 and 2004. Patients. We have allo-geneic 152 patients with relapsed AML (n=110) or ALL (n=42). The medi-an blast count in the marrow was 30% (7-100), and the median blast count in the peripheral blood was 2 (0-99). Median age was 31 (11-62), and the median year of transplant 1995 . Conditioning regimen included total body irradiation (TBI) (10-12 Gy) in 115 patients. The donor was a matched sibling donor (MSD) in 106 , a family mismatched donor (FMD) (n=20) or an unrelated donor (UD) in 26. The graft was T cell depleted (TCD) in 12 cases. Leukemia was diagnosed in first relapse in 42 and more advanced disease, or primary refractory in 110. Results. The overall actuarial survival at 20 years is 15%, the cumulative incidence of transplant mortality (TRM) is 42%, and the CI of relapse related death (RRD) is 48%. There was no impact of stem cell source and no improve-ment of results with time (<= 1995). In multivariate analysis on sur-vival favorable predictors were the use of a donor other than family mismatched (RR 0.45); and bone marrow blast count less than 30% (RR 0.82). The actuarial 20 year survival for 65 patients with both favorable and unfavorable risk factors (FMD vs. UD) was 32% vs. 6%. When looking at patients surviving 100 days, the presence of chronic GVHD was the strongest favorable predictor (RR 0.88, p=0.0008) followed by donor other than family mismatched (RR 0.32, p=0.008), donor age less than 34 years (RR 0.55, p=0.02), and blast count less than 30% (RR 0.58, p=0.07). For 18 patients with all 4 favorable predictors, the actuarial 20 year survival is 54%. Conclusions. This study confirms that 15% of relapsed leukemias can be cured with an allogeneic transplant. The use of young, HLA matched donors and a marrow blast count less than 30% significantly increases the likelihood of long term survival, which is further improved if chronic GVHD develops. This may be relevant when discussing transplant strategies in patients with relapsed leukemia.

**0300**

**TREATMENT OF REFRACTORY CHRONIC GRAFT-VERSUS-HOST DISEASE WITH EXTRACORPOREAL PHOTOPHERESIS**


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Chronic GVHD is the major late complication of allogeneic bone marrow transplant with significant impact on late mortality. Systemic steroids are the first line therapy with only 30-40% complete responses. ECP has been proposed as an alternative therapy for immune-mediated diseases, including transplant rejection and GVHD. Aim of the study.
This is a single Center study testing the efficacy of ECP in patients with steroid-resistant cGVHD. Patients. Twenty-six patients entered this study. Their median age was 40 (range, 5-61) years. The median interval from diagnosis of cGVHD to ECP treatment was 12 (range, 6-168) months. All patients received at least 2 lines of immunosuppression including cyclosporine (CyA) alone, CyA and steroids, CyA and mycophenolate mofetil (MMF), steroids and MMF, steroids and tacrolimus, and ATG. Methods. Patients were treated on 2 consecutive days (one cycle) at 1 week interval for the first month, at 2 weeks interval for the second month and at 4 weeks interval for the subsequent 4 months, for a total of 10 cycles. At 6 months a decision was made whether to continue the ECP treatment as monthly maintenance, depending upon the clinical response. ECP treatment continued to receive the baseline immunosuppressive therapy and were followed in the outpatient clinic. Results. After a median of 15 (range, 7-33) cycles, 11 (76%) of the 14 patients with skin involvement had a partial or complete response. In particular, of 6 patients with severe scleroderma 2 had complete resolution of skin contraction, abrasion and thickening, 2 showed improvement and 2 patients were stable. Of 15 patients with gut involvement, 8 showed a complete response and 3 a partial response. A return to normal values or reduction of abnormal liver function enzymes by at least 50% from baseline were observed in 9 of 12 patients (75%) with liver involvement. Of 9 patients with ocular symptoms due to sicca syndrome, 6 had a complete or partial response. A complete response was observed only in 1 of 3 patients with lung cGVHD. The first signs of response appeared at a median of 3 months. Throughout the ECP treatment course systemic immunosuppressive medication was increased in 4 patients, reduced in 8 and discontinued in 14. The steroid treatment was discontinued at a median of 4.5 months. At a median follow-up of 32 months (range, 11-57), the probability of remaining alive, 3 patients died of cGVHD related infectious complications and 1 of leukemia relapse. Twelve patients (46%) discontinued ECP after a median of 18 (range, 6-24) cycles because of complete and sustained resolution of cGVHD and 5 patients (19%) because of minimal or inadequate response. The ECP treatment is ongoing in 5 patients. The procedures were well tolerated and completed in all cases and no relevant adverse events were observed. Conclusions. Our results confirm the role of ECP in controlling refractory cGVHD. The response of a single organ is independent from the others. The response of liver cGVHD is comparable to mucocutaneous response. New strategies are requested for lung cGVHD.

0301 IMPACT OF DISPARITIES AT SINGLE OR MULTIPLE HLA LOCI ON OUTCOME AFTER UNBILICAL CORD BLOOD TRANSPLANTATION FOR ADULTS WITH HEMATOLOGIC MALIGNANCIES


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Background. The number of HLA disparities considering HLA-A, -B, -C, and -DRB1 is strongly related to engraftment, disease-free survival (DFS), and overall survival (OS) in children undergoing UCBT. The influence of HLA disparities on outcome after UCBT in adults is unknown. Studies lacking.

Methods. To evaluate the possible influence of the number of disparities at single or multiple HLA loci on outcome after UCBT in adults with hematologic malignancies.

Patients and Methods. Patients and Methods. The conditioning regimen was different only in the use of corticosteroids. Patients. Twenty-six patients entered this study. Their median age was 40 (range, 5-61) years. The median interval from diagnosis of cGVHD to ECP treatment was 12 (range, 6-168) months. All patients received at least 2 lines of immunosuppression including cyclosporine (CyA) alone, CyA and steroids, CyA and mycophenolate mofetil (MMF), steroids and MMF, steroids and tacrolimus, and ATG. Methods. Patients were treated on 2 consecutive days (one cycle) at 1 week interval for the first month, at 2 weeks interval for the second month and at 4 weeks interval for the subsequent 4 months, for a total of 10 cycles. At 6 months a decision was made whether to continue the ECP treatment as monthly maintenance, depending upon the clinical response. ECP treatment continued to receive the baseline immunosuppressive therapy and were followed in the outpatient clinic. Results. After a median of 15 (range, 7-33) cycles, 11 (76%) of the 14 patients with skin involvement had a partial or complete response. In particular, of 6 patients with severe scleroderma 2 had complete resolution of skin contraction, abrasion and thickening, 2 showed improvement and 2 patients were stable. Of 15 patients with gut involvement, 8 showed a complete response and 3 a partial response. A return to normal values or reduction of abnormal liver function enzymes by at least 50% from baseline were observed in 9 of 12 patients (75%) with liver involvement. Of 9 patients with ocular symptoms due to sicca syndrome, 6 had a complete or partial response. A complete response was observed only in 1 of 3 patients with lung cGVHD. The first signs of response appeared at a median of 3 months. Throughout the ECP treatment course systemic immunosuppressive medication was increased in 4 patients, reduced in 8 and discontinued in 14. The steroid treatment was discontinued at a median of 4.5 months. At a median follow-up of 32 months (range, 11-57), the probability of remaining alive, 3 patients died of cGVHD related infectious complications and 1 of leukemia relapse. Twelve patients (46%) discontinued ECP after a median of 18 (range, 6-24) cycles because of complete and sustained resolution of cGVHD and 5 patients (19%) because of minimal or inadequate response. The ECP treatment is ongoing in 5 patients. The procedures were well tolerated and completed in all cases and no relevant adverse events were observed. Conclusions. Our results confirm the role of ECP in controlling refractory cGVHD. The response of a single organ is independent from the others. The response of liver cGVHD is comparable to mucocutaneous response. New strategies are requested for lung cGVHD.

0302 CORTICOSTEROIDS FOR PREVENTING GRAFT-VERSUS-HOST DISEASE AFTER MYELOIDAL STEM CELL TRANSPLANTATION: A COMPREHENSIVE META-ANALYSIS

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Background. Corticosteroids after stem-cell transplantation (GvHD) remains a major complication in allogeneic myeloablative stem cell transplantation (SCT) and is considered as the main cause for transplantation related morbidity limiting its wider application. The current standard regimen for preventing GvHD combines cyclosporine (CSA) with a short-course of corticosteroids. The question if the addition of steroids improves patients' outcomes has not been clarified yet as the results of single studies are ambiguous. Aims. To determine the effectiveness of corticosteroids used for the prevention of GvHD after myeloablative SCT in improving overall survival (OS), disease-free survival (DFS), relapse incidence (RI), non-relapse mortality (NRM), acute GvHD grade I-IV, II-IV and III-IV and chronic GvHD. Methods. We conducted a comprehensive literature search in Cochrane Library, EMBASE, MEDLINE, internet databases for ongoing trials, and conference proceedings (1975-2004). Randomised controlled trials evaluating GvHD prophylaxis regimens differing only in the use of corticosteroids were included. A minimum of 75% of the patients undergoing allogeneic myeloablative SCT had to be adults. All authors were asked to provide unpublished and/or missing data. Trial selection, quality assessment and data extraction were done independently by two reviewers. To analyse outcomes with time-to-event data hazard ratios (HR) were calculated on the basis of individual patient data or if not available extracted from the publication using well-established Methods. The weighting was done according to the method of Peto, which assumes a fixed effect model. Heterogeneity of treatment effects between the trials was assessed by using a Chi-squared test with a significance level of p<0.1. Results. 1,709 references were screened, of which 5 randomised controlled trials with 604 patients met the criteria included in the review. The addition of corticosteroids reduced statistically significant the incidence of acute GvHD grade I-IV (HR 0.58, 95% CI 0.45 to 0.76) as well as grade II-IV (HR 0.69, 95% CI 0.51 to 0.92). No significant differences seen for acute GvHD grade III-IV (HR 0.76, 95% CI 0.52 to 1.15) and chronic GvHD (HR 1.21, 95% CI 0.89 to 1.65) as well as no improvements were found for OS (HR 0.99, 95% CI 0.79 to 1.25), DFS (HR 0.95, 95% CI 0.74 to 1.21), RI (HR 0.82, 95% CI 0.57 to 1.18) or NRM (HR 0.88, 95% CI 0.61 to 1.26). Summary/Conclusions. The addition of corticosteroids to GvHD prophylaxis regimens reduces the risk for acute GvHD grade I-IV and II-IV. However, based on the randomised trials currently available there is no evidence that this benefit improves long-term outcomes such as OS, DFS, RI, NRM or chronic GvHD.
Reduced-intensity allogeneic stem cell transplantation (RITA) has emerged as an alternative to myeloablative transplantation in patients with myelodysplastic syndrome (MDS). Given the uncertainty regarding the appropriate conditioning, SFGM-TIC conducted a retrospective multicenter study with the attempt to evaluate the impact of conditioning on patients’ outcome. The record of 61 patients (57 males) with MDS who suffered from cGvHD after RIA was compared with 12 patients after AML before transplantation. Thirty-two patients had RIA at diagnosis, of whom 2 had progressed to RIA-T and 7 to AML before transplantation. Twelve patients had RIA-T at diagnosis and 6 CMML, of whom 8 progressed to AML before transplantation. The median time from diagnosis to RIA was 12 months (6-129). Conditioning regimen consisted of Fludarabin (Flu) plus busulfan (FB; n=29), Flu plus 2-Gy TBI (F-TBI; n=20) and idarubicin plus cytarabine and Flu (Flagida; n=12). Donors were HLA-identical siblings (n=52) and HLA-matched unrelated (n=9). All pts received peripheral blood stem cells. The median of CD34+ infused cell dose was 5x10^6/kg (0.5-17.3). At the reference date of analysis of 1 July 2005, median follow-up was 44.7 months (21-85). Estimated 3-year overall survival (OS), progression free survival (PFS), relapse and transplant-relapse mortality (TRM) were 55%, 79%, 60%, and 19%, respectively. Neither of the 3 conditioning regimens used (FB, F-TBI and Flagida) had impact on patients’ outcome. In multivariable analyses, while acute III/IV grade GVHD development was the only factor found to adversely influencing OS (HR=5.6; 95% CI: 1.1-12.5), chronic GVHD development was the only factor favourably influencing PFS and relapse rate (HR=0.2; 95% CI: 0.1-0.6, respectively). TRM was adversely influenced by male sex of patient (HR=9.2; 95% CI: 1.5-66.6). RIA seems to be an effective treatment in MDS patients irrespective of conditioning type. While acute III/IV grade GVHD appeared to be detrimental, the benefit effect of chronic GVHD was to be bound to GVL effect as demonstrated by the improvement of PFS and relapse rates in patients who developed chronic GVHD. New approaches with focus on immunosuppressive treatment are needed to enhance the GVL effect with an acceptable risk of GVHD.

**Background.** Fasciitis of cGvHD affects predominantly the limbs, but involvement of the trunk leading to respiratory compromise may also occur. Possible associated laboratory features include eosinophilia and elevated anti-nuclear antibodies (ANA). The clinical diagnosis can be confirmed by biopsy and / or magnetic resonance imaging (MRI). Physical therapy to prevent joint contractures is the mainstay of conservative treatment; usually conventional immunosuppressive therapies fail or yield incomplete responses. Extracorporeal photopheresis (or photopherotherapy, ECP) is an attractive alternative for patients with fasciitis of cGvHD because of its systemic action and steroid-sparing effects. Although still not fully understood, tolerance induction through ex vivo psoralen-sensitized and UVA-irradiated T-cells is now believed to be the central mechanism of action of ECP.

**Patients and Methods.** Here we report our seven years experience (2/1999-2/2006) of ECP in 16 consecutive patients with fasciitis of cGvHD: 4 females and 12 males; age 24-63 (median: 44) years; 5 AML, 4 ALL, 3 CMML, 1 MDS and 3 lymphoma patients; 13 with matched related and 3 with matched unrelated donors; 4 patients received RIA conditioning and immunosuppression in 12 with myeloablative conditioning. All patients had severe, extensive cGvHD and MIRI- or biopsy-proven fasciitis. Diagnosis was made 3-28 (median: 15) months after transplantation or donor lymphocyte infusions (DLI), respectively. ECP was performed with the UVAR XTS machine (Therakos, Exton, PA, USA). A total dose of 15, 25 and 30 mJ/cm²/cm² was applied in two to four weeks according to the clinical course. Results. After 4-82 (median: 17) months of ECP therapy, 13 of the 16 patients (81%) had marked (7 patients) or moderate (6 patients) clinical amelioration in their range of movement, whereas 3 patients experienced no change with ECP. Furthermore, all responders were able to considerably reduce (7 patients) or discontinue (6 patients) their immunosuppressive medication. Responses were first noticeable after five to six ECP cycles and continued to improve with maintenance therapy. When a patient’s maximum achievable improvement had been reached, the frequency of ECP cycles could be tapered to once every four to six months, or ECP could be discontinued. ECP was well tolerated without major toxicities or infectious complications. In comparison, among eight historical or contemporaneous control patients treated by standard immunosuppressive agents, who had not received ECP for reasons of patient preference or unsuitable venous access, only two recovered (one with steroids and one with PUVA therapy), whereas five had no change and one deteriorated. Conclusion. In our experience, ECP is a safe and effective immunomodulating approach for patients with fasciitis of cGvHD after allogeneic hematopoietic cell transplantation. Prospective evaluation of ECP in fasciitis of cGvHD in a multicenter trial would be warranted.
resistant to a series of therapies. Thirty-one patients have been already treated by high-dose therapy and PBSCT. Results. Thirty patients (86%) are valuable for response, 6 patients died within 3 months from RIC for causes not related to disease progression (TRM=14%). The response evaluated within 6 months from RIC showed 26 pts in CR (72%) and 10 patients with persistence of disease (28%), 3 relapses (14%), were registered following 12, 18 and 24 months from RIC. Eight patients with disease progression among valuable patients, one patient was alive with stable disease, another patient was alive with chronic graft-versus-host disease (cGVHD) at 18 months from RIC and another patient died from infection and cGVHD at 24 months from RIC. The total number of deaths including TRM was 14%. Five patients are alive with lymphoma and 22 alive in CR (52%) at a mean follow-up of 25 months (7-59 months). The incidence of GVHD was registered in 16 patients (55%) and 6 of them had grade 3-4. This was not correlated to previous therapy or to type of RIC conditioning regimen or to the age of patients. Deaths accounted 11 on 31 (35%) patients who received autologous PBSCT and 4 on 11 (36%) on those who did not.

Conclusions. RIC transplantation provides a high rate of remissions in patients with advanced lymphoma and an acceptable TRM. The results are in line with previous published data: the incidence of GVHD and relapses are not correlated to different conditioning regimen. Future prospective trials including RIC transplantation are planned.

0307
FLUDARABINE BASED REDUCED INTENSITY CONDITIONING FOR ALLOGENEIC TRANSPLANTATION IN PATIENTS WITH NON-MALIGNANT HEMATOLOGICAL DISORDERS
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Patients who are multiply transfused or septic have a poor outcome after allogeneic stem cell transplantation. Seventy patients (53 males and 17 females) with non-malignant disorders underwent allogeneic BMT using a fludarabine based conditioning regimen between 1998 and 2005. The median age was 20 years (range 4-38) and consisted of 25 children and 45 adults. Indications for BMT included severe aplastic anemia (SAAs) in 54, Myelodysplastic syndromes (MDS) in 8, Fanconi’s anemia (FA) in 6 and Thalassaemia in 2 patients. All had 6 antigen matched sibling or family donors. Multiple transfections (>20), sepsis or previous immunosuppressive therapy were considered high risk (HR) and 51 patients (72.8%) were considered high risk patients. The median time from diagnosis to transplant was 16 months (range: 2-108) and the median transplants prior to BMT was 35 (range: 2-80). Conditioning therapy included Fludarabine (Flu) 180 mg/m² over 6 days, Busulfan (Bu) 8 mg/kg over 2 days and ATG 40 mg/kg/day over 4 days (24), Flu 180 mg/m² over 6 days, Cyclophosphamide (Cy) 120 mg/kg over 2 days + ATG 40 mg/kg/day over 4 days (35). Flu 180 mg/m² over 6 days, Cy 20 mg/kg over 2 days + ATG 40 mg/kg/day over 4 days (6), Flu/TBI/OKT3 in 4, Flu/Mel in 1. Graft versus host disease (GVHD) prophylaxis consisted of Cyclosporine alone or in combination with mini methotrexate. Graft source was peripheral blood stem cells in 56 patients and bone marrow in 14. The median cell dose was 5.4×10⁹ MNC/kg (range 2.1-13.6) for PBSCT and 6.2×10⁹ TNC/kg (range 2.1-16) for bone marrow. Nine patients expired within the first 10 days due to sepsis, 59 (96.7%) patients engrafted with a mean time to ANC >500 of 12.2 days (range: 5 - 29), and platelet count >20,000 of 14.2 days (range: 7-30). Two of the patients that had graft failure and expired. Acute GVHD was seen in 18 patients (30.5%) with Grade III-IV GVHD in 6 (10.1%). Chronic GVHD was seen in 14 patients (29.1%) with 9 having limited and 5 with extensive GVHD. Bacterial infections were seen in 16 patients, fungal infections in 19 and CMV in 6 patients. Veno-occlusive disease was seen in 5 patients (7.1%) while hemorrhagic cystitis was seen in 3 (4.2%). Four patients (2 with aplastic anemia and 2 with thalassaemia) had secondary graft rejection. Day 100 mortality was 28% and was related mainly to sepsis. At a median follow up of 20 months (range: 2-84), 46 patients (65.7%) are alive with 44 patients (62.8%) being free of disease. Among patients who were low risk, 17/19 (89.4%) are alive and free of disease. The disease free survival was 66.6% in SAA, 62.5% with MDS, 50% with FA and 0% with Thalassaemia. In conclusion fludarabine based conditioning regimen ensure adequate engraftment with reduced toxicity in high risk patients who are infect- ed or multiply transfused at the time of BMT. Its role even in good risk patients needs to be further explored.

0308
SAFETY AND EFFICACY OF BORTEZOMIB IN RELAPSED MULTIPLE MYELOMA FOLLOWING REDUCED INTENSITY/NONMYELOABLATIVE CONDITIONING AND ALLOGENEIC HAEMATOPOIETIC CELL TRANSPLANTATION

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Background. Despite the promising results obtained in patients with multiple myeloma (MM) receiving a fludarabine based reduced intensity conditioning regimen and allogeneic haematopoietic cell transplantation (HCT), relapse remains an issue. Several clinical trials showed the efficacy of bortezomib in the treatment of refractory/relapsed MM, by inhibition of NF-κB. These findings and the demonstration of the role of NF-κB in the pathophysiology of graft-versus-host disease (GVHD) provide the rational for using bortezomib in patients with MM relapsed after allogeneic HCT. Aims. We evaluated safety and efficacy of bortezomib after reduced intensity/nonmyeloablative conditioning regimen and allografting. Methods. We retrospectively evaluated 24 myeloma patients relapsed after allografting. Conditioning regimens were 2 Gy total body irradiation based in 19 patients, and a combination of thiotepa, cyclophosphamide, and melphalan in 5. Donors were HLA identical siblings in 22 patients, and unrelated in 2. All patients received cyclosporine as part of post-grafting immunosuppression. Bortezomib was administered after a median of 20 months from HCT (range 5-54) and 34 months from diagnosis (range 19-164): 6 patients were in first relapse after HCT, 10 patients in second and 8 beyond the third relapse. Patients received bortezomib 1.0 (n=8) or 1.3 mg/m² (n=16) on day 1, 4, 8, 11 every 3 weeks for a mean of 3 courses (1-7), alone (n=5) or in combination with dexamethasone 20 (n=13) or 40 mg (n=5) on days 1-4 and 15-18 or daily prednisone 75 mg (n=1). No patient was on cyclosporin or thalidomide, and none had active GVHD at the time of administration. Results. Adverse effects were reported in 75% (18/24) of patients: thrombocytopenia was observed in 33% (8/24); >grade 3/5, peripheral neuropathy in 38% (14/24); >grade 3/5, >grade 3/5. Three additional patients experienced grade 2 uticaria, grade 2 liver toxicity and grade 3 neutropenia, respectively. Bortezomib was discontinued after the first cycle in 4 patients due to neurological toxicity, and in 2 patients for disease progression. A dose reduction was required in 5 patients due to neurological toxicity. Flaring of prior chronic limited GVHD was observed in 1 patient who developed mild liver GVHD. After a median follow up of 136 days (range 42-505), 21/24 patients are alive. Two non-responsive patients were responsive post-disease progression. Among patients who completed at least 2 courses, overall response was 67% (12/18) including 5 immunofixation-negative complete remissions. No significant differences in toxicity and response rates were seen between bortezomib plus steroids and bortezomib alone. Conclusions. Bortezomib is capable of inducing disease responses in patients with MM relapsed after allogeneic transplant. No significant effect on GVHD was noted. Interestingly, in this subset we observed a higher incidence of peripheral neuropathy compare to the non-transplant population, which may be related to previous prolonged treatment with high dose cyclosporine. Longer follow up will demonstrate whether remissions will be durable.

0309
CYTKINES AND T-CELL SUBSETS CHANGES IN PATIENTS HAVING GRAFT-VERSUS-HOST DISEASE
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Background. GVHD is the consequence of the activation of donor T-lymphocytes attack the tissue of host. Animal studies strongly suggest that T-cell activation in patients with acute GVHD have a CD4 subset imbalance favoring T helper 1 (Th1), which secrete type 1 cytokines interleukin (IL)-2, IL-12, interferon (INF)-γ, and TNF-α. On the other hand, the polarization toward Th2, which secrete type 2 cytokines IL-4 and IL-10, and subsequent Th2 humoral immune response may be responsible for the development of chronic GVHD. Both Th1 and Th2 are derived from naïve T cell and post clonal differentiation inducers are themselves cytokines: INF-γ and IL-12 for Th1, and IL-4
and IL-10 for Th2. Understanding the cytokines and T cell subsets change in patients with GVHD will theoretically of great help in elucidating the pathophysiology of GVHD. Aim. To see the cytokines and T cell subset changes in patients having acute and/or chronic GVHD. Methods. Consecutive 23 patients received allogeneic hematopoietic stem cell transplantation at China Medical University Hospital were enrolled in this study. 10 mL peripheral blood was collected every 7 days from Day 7 after transplantation till Day 200 (or Day 300 for patients with chronic GVHD). Plasma level of INF-γ, IL-4; IL-10, and IL-12 were determined by ELISA (R&D, Minneapolis, MN, US). Flow cytometric analysis of intracellular INF-γ and IL-4 in mononuclear cells with or without phorbol 12-myristate 13-acetate [PMA] + ionomycin [I] stimulation was used to determine the relative fraction of Th1/Th2 subset. The serial plasma level of each cytokine and relative fraction of Th1/Th2 were then compared to the clinical events in each patient. Results. Plasma IL-10 level increased markedly during period of both acute and chronic GVHD. Plasma INF-γ level also increased in most events of acute and chronic GVHD. With effective immunosuppressive therapy, plasma IL-10 and INF-γ level decreased rapidly. Plasma IL-4 and IL-12 were below the detectable level (0.13 pg/mL and 0.5 pg/mL respectively) in most patients, even during period of severe GVHD. Figure 1 demonstrates the correlation between plasma level of each cytokine (INF-γ, IL-4, IL-10, and IL-12) and clinical course of a patient with both acute and chronic GVHD involving liver. Flow cytometric analysis showed that Th1 (CD4⁺INF-γ⁺) fraction increased markedly within the CD4⁺ T cell population during period of both acute and chronic GVHD. Of great interest is that the CD4⁺INF-γ⁺ T cells can be easily detected in the blood of many patients of GVHD without adding PMA+I to stimulate T cells. Conclusion. Immune reactions in patients of GVHD are much more complicated than in animal models. Type 1 cytokine (INF-γ) and type 2 cytokine (IL-10) may be increased in the blood at the same time during acute and chronic GVHD. Increased Th1 fraction could also be found during both acute and chronic GVHD. Th1 as well as IL-10 and INF-γ may therefore play an important role in the pathogenesis of both acute and chronic GVHD. Besides, they may also be served as good biomarkers in monitoring the clinical course of GVHD.

**Results.** Plasma IL-10 level increased markedly during period of both acute and chronic GVHD. Plasma INF-γ level also increased in most events of acute and chronic GVHD. With effective immunosuppressive therapy, plasma IL-10 and INF-γ level decreased rapidly. Plasma IL-4 and IL-12 were below the detectable level (0.13 pg/mL and 0.5 pg/mL respectively) in most patients, even during period of severe GVHD. Figure 1 demonstrates the correlation between plasma level of each cytokine (INF-γ, IL-4, IL-10, and IL-12) and clinical course of a patient with both acute and chronic GVHD involving liver. Flow cytometric analysis showed that Th1 (CD4⁺INF-γ⁺) fraction increased markedly within the CD4⁺ T cell population during period of both acute and chronic GVHD. Of great interest is that the CD4⁺INF-γ⁺ T cells can be easily detected in the blood of many patients of GVHD without adding PMA+I to stimulate T cells. Conclusion. Immune reactions in patients of GVHD are much more complicated than in animal models. Type 1 cytokine (INF-γ) and type 2 cytokine (IL-10) may be increased in the blood at the same time during acute and chronic GVHD. Increased Th1 fraction could also be found during both acute and chronic GVHD. Th1 as well as IL-10 and INF-γ may therefore play an important role in the pathogenesis of both acute and chronic GVHD. Besides, they may also be served as good biomarkers in monitoring the clinical course of GVHD.

**Figure 1. Cytokines’ change and clinical course of GVHD**
Background. Availability of matched stem cell donor is limiting factor for transplantations to patients who might benefit from this therapy. At most, 25% of patients may be supported by family donors and 30-50% of them receive stem cells from unrelated or other alternative donors. For the remaining patients no suitable donor is available because of unacceptable HLA mismatches. Several approaches are undertaken to increase HLA polymorphism of unrelated donor registries. Preferential typing of ethnic minorities or further typing of donors with rare phenotypes were used optionally. Aims. The aim of this study was to show the structure and genetic differences between urban and rural Polish subpopulations and to check the utility of dispersed rural population for increasing of unrelated donor registry HLA polymorphism. Methods. Five HLA loci (A, Cw, B, DRB1 and DQB1) were DNA typed at allele (4 digit) level in Polish population. The analysis comprised 200 unbiased, healthy individuals living in cities with >100 000 citizens (urban, N=106) and those living out of big cities (rural, N=94). The genetic structure measures of these two Polish subpopulations and five locus phylogenies along with other European, Mediterranean and Far-Eastern populations were analysed. Results. All loci were in Hardy-Weinberg equilibrium in both subpopulations (p>0.05). A significant heterozygote excess was confirmed for DQB1 (p=0.014, SE=0.001) and globally (p=0.026, SE=0.009) in rural sample and on the contrary, global heterozygote deficit was found in urban population (p=0.039, SE=0.012). As could be expected estimates of Nm, the number of migrants exchanged per generation, appeared to be high (Nm=19.60) after correction for sample size (mean sample size, N=100), suggesting high gene flow between two populations caused predominantly by country-to-city migration. Genic and genotypic differentiation tests revealed that none of five loci differentiated the two samples (p=85 and p=82 respectively), confirmed by low Fst values (<0.002). Although similarities between the two samples were obvious, some alleles were met exclusively in one of them. The polymorphism of urban Subpopulation seemed to be slightly higher (121 and 114 HLA alleles in urban and rural population respectively) but we revealed A*2608, 2902, 3301, 6802, Cw*0804, B*0704, 1503, 4501, 4507, 5301, DRB1*0103 and DQB1*0304 only in rural sample. Some allele frequencies (AF) differed significantly between groups. AF of A*2608, 2902, 3301, 6802 and DQB1*0304 were much higher in urban sample (3.7, 6.3, 5.2 and 8.1% respectively) than in rural sample (1.7, 3.0, 2.2 and 3.9% respectively) while those of the B*4402 and DQB1*0603 were much higher in rural sample (6.2 and 8.0% respectively) than in urban sample (6.6 and 3.9% respectively). This heterogeneity did not influence much the phylogenetic analysis, which showed the close relationship of both Polish subpopulations clustering along with Czech population to Slavic branch. Clear-cut departure of Polish subpopulations from Far-Eastern branch and associations with Western-European populations can also be concluded from the neighbour-joining tree. Conclusions. The present study revealed panmictic rather than differentiated structure of urban and rural Polish subpopulations. Nevertheless, rural population remains a reservoir of exclusive genotypes that potentially can increase registry polymorphism.
hematopoietic stem cell (HSC) recruitment has an influence on transplant outcome after reduced intensity allogeneic peripheral blood stem cell transplantation (PBSCT): a study of the societe francaise de greffe de moelle osseuse et de therapie cellulaire (SGFM-TC) registry

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Impact of graft product on transplant outcome after PBSCT is actually demonstrated. We investigated retrospectively the potential impact of HSC recruitment procedure (i.e. G-CSF stimulation schedule and apheresis number) and graft composition (CD34+ and CD3+ cell number) on transplant outcome (GVHD, OS, EFS). Our analysis concerned 488 HLA matched sibling allogenic reduced intensity conditioning (RIC) PBSCT for haematologic malignancies (116 MM, 110 AML, 109 NHL, 41 CLL, 41 MDS, 24 CML, 19 HD, 17 ALL and 11 MPS) reported on the SGFM-TC registry between 1998 and 2004. RIC-PBSCT was performed during first line treatment in 225 (49%) patients and a previous HSCCT was recorded in 35% of the cases. Before RIC, 161 patients were in Complete Response, 34 in Stable Disease and 132 in Progression Disease. Follow-up was updated in April 2005. G-CSF medi- um duration was 5 days (3-7days) at a median dose of 10µg/kg/day (4.6-16). G-CSF was given in 40% of the stimulations. Filgrastim was used in 59% of the donors (Lenograstim: 41%). Only 107 donors (22%) had a single apheresis. The median number of CD34+ cells infused was 5.6×10^6/CD34/kg (1.2-26) and the median CD3+ cells was 302×10^3/CD3+/kg (63-996). Conditioning regimen was most frequently an association of Fludarabine Busulfan and Anti Thymocyte Globuline (246 cases, duration of ATG 1 day: 18%; 2 days: 20%; 3 days: 20%; 4 days: 8%; 5 days: 88%); or Fludarabine + TBI 2 Gy (125 patients). GVHD prophylaxis was a cyclosporine based treatment in 478 (95%) patients. Medi-an follow-up after transplantation was 35 months (range: 0-86). Acute GVHD (grade II-IV) and cGVHD incidences were 35% (n=163) and 50% (n=217 for 480 patients) respectively. The 3-year OS was 40% and the 3-year EFS was 54%. Treatment related mortality was 15% at 3 years. In multivariate analysis studying pre and post transplant factors a significant impact was shown of G-CSF duration (HR: 0.79 (0.62-1)p=0.05). G-CSF daily dose (HR: 1.13 (1.12-1.12)p=0.04) on OS and a trend for G-CSF dose on EFS (HR: 1.12 (0.97-1.25)p=0.12). Other variables also influenced OS (NHL vs AML, aGVHD grade II vs 0-I and III-IV vs 0-I and cGVHD: yes vs no) and on EFS (Sex mismatch, ABO incompatibility, NHL vs AML, FBS ATG duration: 5 days vs 2 days, ac-GVHD grade II vs 0-I and III-IV vs 0-I and cGVHD: yes vs no). No influence of graft composition or stem cell recruitment was demonstrated on incidence and severity of aGVHD and cGVHD although we found a significant impact of conditioning (FBS ATG 1 day vs 2 days and Fluda-TBI vs FBS ATG 2days). In conclusion, our demonstration that graft composition and conditioning have no impact on transplant outcome. Prolonged administration of moderate dose of G-CSF seems to be the best schedule for PBSCT recruit- ment.

Comparative outcomes of fludarabine-based nonablative and ablative conditioning for patients with advanced hematologic malignancies

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Background. The role of nonablative allogeneic transplantation is not clearly defined for advanced hematologic malignancies. Aims. We have conducted a comparison of the outcomes of nonablative and ablative conditioning directly for the treatment of patients suffering from advanced hematological malignancies. Methods. Adult patients with advanced hematologic malignancies who underwent allogeneic HSCT: AML (n=56); chronic myeloid leukemia beyond 1st chronic phase, 6; refractory Non-Hodgkin's lymphoma, 10; refractory multiple myeloma, 3) received transplants from human leukocyte antigen-matched donors, either related or unrelated, coupled with either nonablative (n=40; fludarabine/melphalan, 28; fludarabine/cyclophosphamide, 12) or ablative conditioning (n=55, busulfan/cyclophosphamide). The patients receiving nonablative conditioning were elderly, or exhibited contraindications for ablative conditioning. Results. Neutrophil engraftment (i.e., time to ANC>0.5×10^9/L) occurred more rapidly in the nonablative group (median, 9 days; range, 0-19 days) than in the ablative group (median, 18 days; range, 11-38 days)(p<0.001). The time required to achieve a platelet count in excess of 20×10^9/L was 12 days (median; range, 7-28 days) in the nonablative group, and 22 days (median; range, 9-64 days) in the ablative group (p=0.001). Acute graft-versus-host disease (grade II) occurred at comparable frequencies in the nonablative and ablative groups (25% and 26%). Hepatic veno-occlusive disease developed in 1 patient (3%) in the nonablative group, and 7 patients (20%) in the ablative group (p=0.02). Day-100 and 1-year NRMs were 33% and 47% in the nonablative group patients, as compared with 38% and 56% in the ablative group patients (p=0.68). The overall 1-year survival rates of the nonablative and ablative group patients were 44% and 15%, respectively (p=0.04). Conclusions. We noted a clear trend towards a more favorable overall survival rate in the nonablative group patients. The results of this study indicate that patients suffering from advanced hematological malignancies might benefit from treatment via nonablative transplantation.
Background. We evaluated haematological and immunological characteristics of four thalassemia patients after T-cell-depleted HLA-haploidentical stem cell transplantation. Methods. We evaluated the clonogenic capability by the colony forming cell assay (CFC) and the long term culture-initiating cell (LTC-IC) assay at baseline and 20 days after transplant. Stromal cells were obtained from long term culture of bone marrow mononuclear cells (BM-MCs) and analysed by immunohistochemistry. Lymphocyte subsets were studied by flow cytometry; and stromal IL-7 production by BM-MCs was analysed by ELISA. Results: At baseline, no significant differences were observed in haematological and in immunological parameters in thalassemia patients when compared with a group of normal subjects. Day +20 after transplant, a reduced clonogenic capability was observed (4±2 vs. 41±40 CFU-E, 17±9 vs. 109±22 BFU-E, 3±1 vs. 9±6 CFU-GE/MM and 16±10 vs. 66±23 CFU-GM). The number of primitive bone marrow (BM) progenitor cells was also decreased (1.5±1.4 vs. 15.4±3.6 LTC-IC/F106 BM-MCs). In addition, stromal cells secreted lower IL-7 levels (0.3±0.1 pg/mL vs. 0.8±0.1 pg/mL, in controls) and displayed by immunohistochemistry an altered phenotype. Upon light microscopy examination, the majority (75%) of these cells appeared as moderately large cells, frequently rounded, with a spindle shape and branching cytoplasmic processes (fibroblast-like). Compared with normal subjects, thalassemia patients showed: reduction of naive CD4+ T-cells (2±0.5% vs 50±10%), reduction of thymic naive CD4+ T-cells (1±0.2% vs 40±12%), and a significant increase of CD4+ cells activation markers (CD95, HLA-DR and CCR5). IL-7 receptor (CD127) expression was also significantly decreased on CD4+ T-cells and on naive CD4+ T-cells (CD4+/CD45RA+CD62L+/CD127+) NK cells were among the first lymphocytes to repopulate the peripheral blood, and up to 70% of these cells were CD56 bright whereas CD16+ NK cells were decreased. Conclusions. Twenty days post transplant, an impaired growth and differentiation capacity of stem/progenitor cells were observed in thalassemia patients, in parallel with an altered hematopoietic stem/progenitor cells. CD56+ NK cells develop more rapidly than other lymphocytes, but CD16+ NK cells (with cytotoxic potential) require more prolonged exposure to maturation factors (IL-2) in the bone marrow. An IL-7/IL7R pathway dysregulation has been also observed, possibly involving bone marrow stromal cells. In vitro studies are ongoing about the use of cytokines (IL-2, IL-7, IL-2 plus IL-7) supporting T-cell development.

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Background. Corticosteroid-refractory GvHD is difficult to manage, and is associated with high morbidity and mortality. Cyclophosphamide (Cy) is an established immunosuppressive and cytotoxic drug widely used as a part of conditioning regimens. Pulse Cy in the GvHD treatment is based on the Cy efficiency for the treatment of many autoimmune disorders and the autoimmune nature of GvHD. In our previous work, we showed that intestinal GvHD responded poorly to pulse Cy, whilst liver, skin and oral GvHD responded well. The liver GvHD is more frequent than other GvHD forms. Aims. We used pulse Cy in the treatment of corticosteroid-refractory liver GvHD with aims to evaluate efficacy, toxicity and influence of Cy to some clinically significant parameters. We analyzed our new data concerning liver GvHD. Methods. This is a retrospective study of 20 patients (pts) with hematological malignancies after allogeneic peripheral blood stem cell transplantation: 12 pts had acute GvHD (2 pts grade I, 3 pts grade II, 7 pts grade III), 4 pts had chronic extensive GvHD and 4 pts developed liver GvHD upon DLI. Three pts had only liver GvHD, 17 pts had GvHD with involvement of liver and/or oral mucosa, skin, gut. Nine patients had hepatic variant of liver GvHD (serum aminotransferase ALT or AST elevation above 10 times the upper normal limit). All patients were treated by cyclosporine A and steroids in dose 2 mg/kg before pulse Cy, six patients had another previous therapy (mycophenolate mofetil, tacrolimus, ATG, alemtuzumab). Steroid-refractory GvHD was defined as lack or response to steroids administered for at least 5 consecutive days. Twenty pts with corticosteroid-refractory liver GvHD were treated by Cy at median dose of 1g/m2 (range 460 mg/m2-1500 mg/m2). Sixteen patients received one pulse Cy, 4 patients two pulses of Cy. Results. There were 55% CR (11/20), 10% PR (2/20) and 35% NR (7/20). However, in 8 pts with NR their clinical status stabilized and they responded to another treatment. Eight pts (89%) from nine pts with hepatic variant of liver GvHD reached CR. Five pts died, 3 from intractable liver and intestinal GvHD, 1 from intestinal GvHD with liver GvHD in PR, and 1 from relaps of leukemia. No influence of pulse Cy to chimerism and disease status was observed. Leukopenia and/or thrombocytopenia WHO grade 4 developed in 5 patients. When myelosupression appeared, it was usually short-lived (1-4 days). Twelve infectious complications occurred in 8 of 20 pts (pneumonia 2x, febrile neutropenia 1x, CMV positivity 6x, BKV positivity 3x), all of them resolved after antimicrobial therapy. No other significance toxicity after Cy pulse was observed. Overall survival is 75%, with median and maximum follow-up of 12 and 88 months, respectively.

Conclusions. Pulse Cy has a good toxicity profile and the cost of the drug is negligible. According to our results, pulse Cy is very effective therapy of steroid-refractory liver GvHD.
Apoptosis / Transcriptional control / Signalling

HALOFUGINONE, INHIBITOR OF TRANSFORMING GROWTH FACTOR (TGF)b, INDUCES APOPTOSIS AND CELL CYCLE ARREST OF MULTIPLE MYELOMA CELLS IN VITRO AND IMPROVES HIND LIMB PARALYSIS IN THE ST2 MM MOUSE MODEL IN VIVO

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Multiple myeloma (MM) is a devastating malignancy which remains incurable despite recent novel therapeutic compounds. It was previously shown that Activin A, a member of the transforming growth factor (TGF)b superfamily, is a potent inhibitor of myeloma cells that act by blocking the cell cycle and inducing apoptosis. Halofuginone is a new novel inhibitor of TGFb signaling that works by inhibiting Smad3 phosphorylation. The purpose of this study was therefore to assess the effect of Halofuginone on MM cell lines in vitro and to evaluate its putative therapeutic potential using the mouse ST2 MM tumor model that mimics arrest and cell death in Halofuginone treated MM cells. Finally, shrankage, chromatin condensation, nuclear and DNA fragmentation death of the MM cell lines in a dose dependent manner (IC50 varied...}

DOWNREGULATION OF RXRα EXPRESSION IS ESSENTIAL FOR THE DIFFERENTIATION AND PROLIFERATION OF NEUTROPHIL GRANULOCYTES

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Neutrophil granulocytes are short-lived leukocytes that have to be constantly regenerated from myeloid progenitors. Retinoid-X-receptor-α (RXRa) is the predominant RXR protein in myeloid cells. RXRa is able to heterodimerize with other nuclear receptor (NR) family members or form signal-competent homodimers. RXRa partner availability regulated by intracellular RXRa abundance is thought to determine NR signaling. However its regulation in primary neutrophil versus monocyte differentiation remained uncharacterized. Here we show that myeloid progenitors express RXRa protein at sustained high levels during M-CSF-induced monopoiesis. In sharp contrast, RXRa is downregulated during G-CSF-induced late-stage neutrophil differentiation. Ectopic RXRa inhibited G-CSF-dependent cell proliferation of granulocyte progenitors as well as their differentiation to late stage CFU-L+ neutrophils in a serum-free culture model of CD34+ human progenitors. Furthermore, ectopic RXRa was sufficient to redirect G-CSF stimulated progenitors to monocytes. In line with its elevation in monocytes, RXRa failed to inhibit, but rather augmented M-CSF-dependent monocyte generation. Functional genetic interference with RXRa signaling in hematopoietic progenitor/stem cells using a dominant-negative RXRa promoted the generation of late stage granulocytes in vivo and in vitro. Therefore, downregulation of RXRa protein is required for neutrophil generation. This differential regulation of RXRa protein expression is determined by granulocyte versus monocyte cytokine signals.

TRAIL-R3 EXPRESSION ON MYELOID LEUKEMIC BLASTS IS RELATED TO SHORTENED OVERALL SURVIVAL

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Background. Since chemotherapy and transplantation can cure only around 35% of patients with acute myeloid leukemia (AML), there is still need for complementary and targeted treatment modalities. One of them could be the use of TNF-related apoptosis-inducing ligand (TRAIL). TRAIL is expressed by effector T cells and induces apoptosis via the death receptor intrinsic pathway. Activation of this pathway in addition to the mitochondrial pathway (by chemotherapy) has synergistic effects in vitro. In human, 4 membrane bound receptors have been identified: two of them (TRAIL-R1 (R1) and TRAIL-R2 (R2)) contain a functional death domain and are capable of starting the apoptotic cascade, and two others (TRAIL-R3 (R3) and TRAIL-R4 (R4)) lack a functional death domain and function as decoy receptors. Most normal cells express R3 and R4, whereas many tumor cells express R1 and R2. This makes soluble recombinant TRAIL an attractive candidate for targeted therapy; phase I clinical studies for solid tumors are launched. Until now, sparse data on TRAIL sensitivity of myeloid leukemic cells have demonstrated low TRAIL sensitivity. Aims. Investigation of possible role for TRAIL treatment in AML patients. Methods. We investigated blood and bone-marrow samples of 113 patients with AML for TRAIL receptor expression by flow-cytometry. Results were correlated to clinical data. Four myeloid leukemic cell lines with different expression levels of TRAIL receptors were tested for TRAIL sensitivity by treatment with soluble TRAIL. Downregulation of R3 expression was performed by treatment PI-PLC and cyclohexamide. Results. In contrast with published data, we found surprisingly (pro-apoptotic) R1 and R2 expression (mean percentage positive cells 16% and 54%, range 0-79% and 0-97% respectively) versus R3 and R4 expression (mean 9% and 10%, range 0-71% an 0-45%) indicating a TRAIL sensitive profile for myeloid blasts. Surprisingly, the expression of the anti-apoptotic R3 strongly correlated to survival. Expression of >25% blasts positive for R3 resulted in shortened overall survival (p=0.0051), see figure 1. In multivariate analysis R3 expression remained a significant prognostic factor next to cytogenetics (p=0.03 and p=0.015 respectively). In vitro studies on myeloid leukemic cell lines confirmed TRAIL sensitivity in cells that expressed R1 and R2. Furthermore, simultaneous expression of R3 clearly lowered the amount of apoptosis, suggesting that TRAIL effects are inhibited by binding to R3. Removal of R3 by treatment with PI-PLC resulted in 50% reduction of R3 expression and partially restored TRAIL sensitivity in vitro. Conclusions. Our data suggest that, in contrast to earlier reports, there might be a role for TRAIL in apoptosis induction of AML blasts. R3 expression is a strong predictor for overall survival. In AML cell lines R3 expression resulted in less TRAIL sensitivity and removal of R3 partially restored TRAIL sensitivity. Modulation of R3 might yield additional new therapeutic options for AML patients.
**Background.** HTLV-1 is the etiologic agent of adult T-cell leukemia/lymphoma (ATLL). In *vivo*, HTLV-1 infects both CD4+ and CD8+ lymphocytes, yet induces ATLL that is regularly of the CD4+ phenotype. Aims: To compare infection of CD4+ and CD8+ T cells by HTLV-1 *in vivo* and *ex vivo* in carriers without malignancy, and its implication in genesis of ATLL. Methods. *In vivo*: comparative analysis of proviral loads (real-time quantitative PCR) and clonality pattern (Inverse PCR) of highly purified CD4+ and CD8+ infected cells from 10 patients without malignancy. *ex vivo*: comparative analysis of 66 clones (infected versus uninfected / CD4+ versus CD8+) generated by limiting dilution from 4 infected patients. Monoclonality was confirmed by analysis of TCR-γ chain gene rearrangements of each clone (multiplex PCR-γ denaturating gradient gel electrophoresis analysis). Studied parameters: cell proliferation (cell count and 3H-thymidine incorporation, with and without interleukine-2), cell cycle (measurement of DNA content by flow cytometry after propidium iodide [PI] staining), apoptosis (flow cytometry after annexin V and PI staining), viral expression (ELISA and real-time quantitative RT-PCR) and cytology.

**Aim:** To characterize this breakpoint region in order to identify regions and factors responsible for ectopic homeobox gene activation. Methods. We used cytogenetic, molecular and bioinformatic Methods. Fluorescence in situ hybridization (FISH), fiber-FISH and Halo-FISH; RT- and RQ-PCR, DNA-transfer by electroporation and lentivirus, gene expression knockdown by antisense oligos or siRNA, chromatin immunoprecipitation (ChIP); TRANSFAC database. Results. Using the TRANSFAC database we designed 26-mer double-stranded oligos (DSO) to constrain DNase-I hypersensitive sites (DHS) and other putative regulatory sites within the downstream BCL11B non-coding region by transfection of a T-ALL cell line (PEER) in which NKX2-5 is activated by inversion downstream of BCL11B. NKX2-5 modulation was regionally dependent, with 8/9 inhibitory DSO lying telomeric of the 14q32 breakpoint corresponding to regions displaying tight nuclear matrix attachment. DSO neighboring BCL11B itself inhibited that gene only, while the 4 DSO most efficacious against NKX2-5 lay adjacent to its insertion point at 14q32, matching both orphan DHS clusters and a desert acetylation island. ChIP analysis showed neither NKX2-5 nor TLX3 to be promoter-acetylated in T-ALL cells, unlike regions at efficacious inhibitory DSO. Expression of NKX2-5 and TLX3 in T-ALL cells was, nevertheless, sensitive to histone deacetylation inhibition implying their extrinsic regulation by acetylated factors. Gene knockdown studies identified PU.1 which is known to be regulated by acetylation as a key factor activating ectopic homeobox gene expression in T-ALL cells. TRANSFAC analysis revealed HMGA1 binding sites predominantly located near inhibitory DSO sequences. We discuss these results in the context of published interaction between PU.1 and HMGA1 and involvement of HMGA1 in enhancerosome structure/function. Summary/Conclusion. We have identified a distal leukemogenic regulatory hotspot downstream of BCL11B to serve as a potential therapeutic target within ‘junk’ DNA.

**References.**

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**Background.** Spred1 proteins are inducible inhibitors of signalling induced by receptor tyrosine kinases. They are implicated in negative feedback interactions that regulate intracellular pathways. The repressive function of Spred proteins targets several TK receptors so resulting in a variety of biological effects. Spred proteins, after growth factor stimulation, translocate to the plasma membrane, become tyrosine phosphorylated and interact with components of the Ras/MAPK and Ras/Raf/Erk pathways. Aim: The aim of this study was to assess whether the activation of Bcr-Abl pathway leading to the disruption of many biological processes, could be supported by a defective signalling inhibition. Methods. Using a Real Time PCR we studied the expression lev-
el of Spred1 in 80 samples collected from CML patients at diagnosis (15 PB and 65 BM), and 9 BM samples from patients in blast phase (BC). Furthermore, 12 CP patients were evaluated also at the time of the achievement of complete cytogenetic remission. Finally, 36 normal controls (20 PB and 16 BM) were studied. The protein level was analyzed by western blot and immunofluorescence assay. Sequence analysis of the coding and promoter regions was performed. In order to establish the effects induced by the absence of Spred1 on proliferation, we transfected K562 cells with Spred1 plasmid. After transfection colony growth was evaluated in semised medium, the proliferation rate was estimated by MTT assay and by the incorporation of 3H thymidine. Results. We found that Spred1 transcript amount is significant reduced in CP CML samples (mean value of 2±0.02; range 0.1-0.002) when compare to normal controls (mean 2.4) with a p value of 0.000002. This difference is even more sound in BC CML cells where Spred1 transcript is 4 logs lower compared to normal controls (2±0.005; range 0.0000001). The expression levels significantly increased after reaching the cytogenetic remission (mean value of 2±0.09; p=0.00009). W

Results. This study clearly demonstrates that the absence of Spred1 protein, a physiological inhibitor of RTK mediated signalling, is a common finding in CML cells and this may support the abnormal proliferation in Bcr-Abl positive cells.

0324 ROLE OF ID AND HES PROTEINS IN ACUTE PROMYELOCYTIC LEUKEMIA


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Acute promyelocytic leukemia (APL) is uniquely sensitive to treatment with all-trans retinoic acid (ATRA), which overcomes the differentiation arrest and induces terminal granulocytic differentiation of the leukemic blasts. In 98% of the cases of APL, the leukemic cells express a promyelocytic leukemia (PML)-retinoic acid receptor (RARβ) fusion protein as a result of a t(15;17) chromosome translocation. Previously, we have identified ID1 and ID2 as direct retinoic acid target genes. These proteins act as antagonists of basic helix-loop-helix (bHLH) transcription factors. ATRA induced a rapid, transient increase in ID1 and a sustained upregulation of ID2 both in the APL cell line NB4 as well as in primary leukemia cells from APL patients. To assess the relevance of this upregulation, ID1 and ID2 were overexpressed in NB4 cells. Overexpression inhibited proliferation and induced a G0/G1 accumulation. These results indicate that ID1 and ID2 are important retinoic acid responsive genes in APL. In addition, we studied another group of antagonists of bHLH transcription factors, the Hairy and Enhancer of split (HES) genes. We identified HES1, which is involved in Notch signalling, and has a very similar biochemical function as the ID-proteins, as a direct ATRA-responsive gene. In NB4 cells and in APL patient cells, ATRA induced a rapid but transient increase in HES1 followed by a sustained downregulation of HES1 expression. In the 5’ upstream promoter we identified a retinoic acid response element. Chromatin-immunoprecipitation assays revealed an interaction of PML-RARA with the HES1 promoter, suggesting a role for HES1 during ATRA-induced differentiation of APL cells. Overexpression of HES1 in APL cells will provide insight into the function of HES1 during APL cell proliferation and differentiation, and apoptosis.

0325 REGULATION OF AUTOPHAGIC PROGRAMMED CELL DEATH BY THE BALANCE BETWEEN CERAMIDE AND SPHINGOSINE-1-PHOSPHATE THROUGH MAMMALIAN TARGET OF RAPAMYCIN (mTOR) IN HUMAN LEUKEMA HL-60 CELLS

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Background and Aim. The balance between ceramide and sphingosine-1-phosphate has been suggested to be critical to cell death and survival in the fate of leukemia cells. Autophagy is recognized as one of the important mechanisms in the metabolism of cellular components, and has recently emerged as a cytotoxic-independent programmed cell death (PCD) system different from classic apoptosis. Unlike apoptotic PCD, the role of ceramide and sphingosine-1-phosphate (SIP) in amino acids deprivation (AA(-))-induced autophagic PCD remains unclear. So, in this study, we examined the role of ceramide and SIP on the induction of autophagy and autophagic PCD.

Methods. Human leukemia HL-60 cells were cultured in RPMI 1640 medium containing heat-inactivated 10% fetal bovine serum and transferred to AA(-) medium for induction of autophagy. Autophagy was assessed by the autolysosome fluorescent drug monodansylcadaverine (MDC), electronmicroscopy and cleavage of MAP-LC3 from 18 to 16kDa. Apoptosis was judged by nuclear DNA fragmentation. The synthesis of autophagy-related proteins was assessed by in vitro kinase assay based on the levels of phosphorylation of 4E-BF and p70S6 kinase. The expression plasmid constructs used were the constructs for constitutively active mTOR kinase and kinase-dead mTOR kinase, which have been previously described. HL-60 cells were transiently transfected by the electroporation method using NucleofectorTM kit (Amaxa Biosystems). Results. The generation of intracellular ceramide precedes AA(-)-induced autophagy and subsequent PCD in a caspase-8-independent manner in human leukemia HL-60 cells. SIP inhibits AA(-)- or N-acetylsphingosine (C2-ceramide)-induced autophagy through activation of mTOR, as judged by phosphorylation of 4E-BF and p70S6 kinase. In contrast, C2-ceramide overcomes SIP-inhibited induction of autophagy by inhibiting mTOR. Genetically overexpressed mTOR inhibits AA(-)- or C2-ceramide-induced increase of autophagy with activation of MAP-LCS, a mammalian homologue of yeast Apg8/Aat7, whereas overexpression of kinase-dead mTOR blocks the inhibitory effects of SIP on induction of autophagy by inhibition of MAP-LCS activation. Conclusions. We here show that ceramide and SIP play an exclusive role on the induction of autophagy and autophagic PCD through the regulation of mTOR-dependent MAP-LCS.

0326 THE MECHANISMS UNDERLYING THE CYTOTOXIC EFFECT OF CDK INHIBITOR (ROSCOVITINE) ON LEUKEMIC CELL LINES

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Background. Roscovitine is a 2,6,9-trisubstituted aminooaruridine analogue that compete with ATP for binding to the active site on Cyclin-dependent kinases (CDKs). It inhibits CDK2/cyclinε, CDK7/cyclinH and CDK9/cyclinT. The cytotoxic effect of roscovitine and its analogues has been reported in several cancer cell lines in vitro and in animal models of cancer xenografts in vivo. The phase II clinical trials in lung and breast cancer and phase I trial in gliomerulonephritis are currently ongoing. Aim. We have studied the mechanisms of roscovitine-induced cytotoxicity and cell death in leukemic cell lines HL60 (myeloid), Jurkat and K562 cells were cultured in RPMI1640 supplemented with 10% FBS. Cells were treated with Roscovitine in concentrations of 5 μM, 25 μM and 50 μM up to 48 hours. The cells were examined for viability using trypan blue exclusion assay, proliferation using 5H-thymidine incorporation assay, apoptosis using morphological criteria in Giemsa staining, cell cycle using propidium iodide and flow cytometry. Specific proteins were detected by Western blotting. Results. Cytotoxic effect of roscovitine expressed as decrease in viability and proliferation was concentration- and time dependent in HL60 and Jurkat cells. In contrast, no remarkable effect on K562 cells was observed. Apoptotic morphology was firstly observed 5h after the treatment with roscovitine and markedly increased 6h in HL60 and Jurkat cells, but not in K562 cells. In HL60 and Jurkat cells, the cell cycle analysis has shown an increase in sub-G1 cells at 6 hours with maximum at 24h without preceding cell cycle arrest. In K562 cells sub-G1 peak increased subsequently to G2/M arrest. In HL60 cells, cleaved fragment of caspase 2 was found from 6 hours of incubation with Roscovitine.
Activated fragments of caspases 3, 7 and 9 were observed at the same time point. Poly(ADP-ribose) polymerase (PARP) was cleaved to 89kDa, confirming caspase-3 activation. In the mitochondrial pathway, Bcl-2 was cleaved to 23kDa and release of cytochrome c and AIF were observed. Activated fragment of caspase 8 was observed at 24 hours in Roscovitine 50uM. In Jurkat cells, caspase 2 was cleaved later than in HL60 (24 hours), while caspase 8 at 6 hours. Caspase 3, 7, and 9 were cleaved similarly as in HL60 cells. Release of cytochrome c and AIF from mitochondria at 6 hours was detected. However, Bcl-2 was not activated. In K562, no caspase activation was detected at studied time points.

Conclusion. Roscovitine has shown a potent cytotoxic effect in both HL60 and Jurkat cells, whereas K562 has been resistant. Caspase 2 is involved in DNA damage and cytochrome c release in HL60. Apoptosis is induced by caspase 8 activation and mediated by mitochondrial pathway in Jurkat cell line. K562 cells are resistant to Roscovitine that maybe due to Bcr/Abl gene and loss of p53 function.

0328

METHYLATION-ASSOCIATED TRANSCRIPTIONAL SILENCING OF THE C/EBP α GENE IN ACUTE MYELOGENOUS LEUKEMIA

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Background. A regulatory network including various transcription factors controls the differentiation of hematopoietic stem cells and progenitor cells. The CCAAT/enhancer binding protein α (C/EBPα) is a transcription factor implicated in the regulation of myelopoiesis that plays an important role in the coordination of cellular differentiation with apoptosis. Specific point mutations of C/EBPα have been reported in acute myelogenous leukemia (AML). Mutated forms of C/EBPα may impair granulocytic differentiation and thus contribute to leukemogenesis. Aims. Aberrant CpG island methylation in association with transcriptional silencing has been recognized to act as an alternative to mutations and deletions to disrupt tumor suppressor gene function. A large number of genes involved in fundamental cellular pathways have been shown to be affected by this epigenetic phenomenon. In this study, we investigated the possible role of CpG island hypermethylation in the transcriptional regulation of the C/EBPα gene in AML. Methods. Aberrant methylation of C/EBPα in hematopoietic cell lines and patient samples were assessed by methylation-specific PCR (MSP). Methylation patterns in cell lines were further analyzed in detail by bisulfite sequencing. Expression of C/EBPα was determined by real time reverse transcription PCR. Results. In hematopoietic tumor cell lines, aberrant methylation of the C/EBPα promoter region was associated with transcriptional silencing. Treatment of cell lines, which carry a hypermethylated C/EBPα gene, with the demethylating agent 5-aza-2'-deoxycytidine resulted in C/EBPα reexpression. In the cell lines L540 and Raji, bisulfite sequencing of individual alleles revealed dense methylation throughout the region around the transcription start site, while HL-60 cells were almost completely unmethylated. The analysis of diagnostic bone marrow and blood specimens from adult patients with AML by MSP showed aberrant methylation of the C/EBPα promoter region in 12/69 (17.4%) samples. Hypermethylation of C/EBPα in AML could be detected in all cytogenetic risk groups, but was restricted to the French-American-British (FAB) subtypes M2, M4 and M5. There was a trend towards a better overall survival in AML cases with C/EBPα hypermethylation. Summary. These data indicate that hypermethylation of the transcription factor C/EBPα is a common epigenetic event in adult AML. Hypermethylation-associated silencing of C/EBPα may, in addition to genetic aberrations, interfere with the cellular differentiation process and thus contribute to the malignant phenotype. The exploration of our growing knowledge about epigenetic aberrations in hematopoiesis may help develop novel strategies in diagnosis and treatment of AML for the future.

0329

ROLE OF SIGNAL TRANSDUCTION PATHWAYS AND THE MICRONENVIRONMENT IN THE EMERGENCE OF MINIMAL RESIDUAL DISEASE IN ACUTE MYELOID LEUKEMIA


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Background. Relapse is common in patients with acute myeloid leukemia (AML) due to the emergence and outgrowth of minimal residual disease (MRD). High frequency of flow cytometric (FACS) detected MRD identifies patients with high risk of relapse (Feller et al., Leukemia 2002; 16: 181-188). New treatments, such as the use of demethylating agents, have been reported to eradicate these MRD cells in order to improve survival. Aims. Our research focuses on how aberrant signal transduction, e.g. constitutive phospho-AKT (pAKT) and phospho-ERK (pERK) expression and Nuclear Factor kappa B (NFκB) activity, all in interaction with the bone marrow microenvironment (BM-ME) contribute to the emergence, persistence and outgrowth of MRD and thereby effect prognosis of the patient. Methods. A sensitive and reproducible FACS assay was developed for the quantification of phosphorylated protein expression in AML. Results. Good correlations were found between the FACS assay and Western Blot- and ELISA techniques in both cell lines and patient samples. Specific pharmacological signals was proven using the inhibitors LY294002, for PI3K-dep endent AKT phosphorylation, U0126 for MAPK dependent ERK phosphorylation, and MG132 a proteasome inhibitor for NFκB activity. Using a NFκB activity ELISA both a cell line (HL60) and patient samples (n=5) showed NFκB activity that was upregulated by adherence to...
fibronectin to stimulate the BM-ME factor. In HL60 and 1.5 in 3/4 patient samples, with no or less upregulation in non-adherent cells. Response of pAKT, pERK and pNFκB in reaction to fibronectin binding is under investigation using firstly Western Blot but eventually using our FACS assay after optimisation for the BM-ME conditions. Subsequently, the FACS assay was adapted to study AML subsets, in particular stem cells (CD34+CD38+) and MRD cells. pAKT, pERK and pNFκB expression could be shown in the CD34+CD38- stem cells. Also the expression of pAKT, pERK and pNFκB in subpopulations with aberrant immunophenotypes (e.g. CD34+CD7+, CD34+CD56+) enables the comparison with signal transduction in MRD cells identified by these aberrancies. Summary/Conclusions. AKT, ERK and NFκB signaling can now be studied in subpopulations highly relevant for clinical outcome, i.e. stem cells, MRD cells and MRD stem cells. We have shown how to detect stem cells under MRD conditions (van Rhenen et al. Blood 2005; 106; 4, abstract van Rhenen et al. this conference). In particular changes of signaling in the course of disease, as may be inferred from reported FLT3 ITD changes from diagnosis to relapse, unveils the possibility to study signal transduction under MRD conditions. This and the study of the interactions between leukemic cells and the microenvironment contributing to persistence and outgrowth of MRD might ultimately guide the development of new treatment strategies, directed at the MRD (stem) cell and/or the microenvironment.

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**0330**

**TRANSIENT POST-TRANSLATIONAL UPREGULATION OF TELOMERASE ACTIVITY DURING MEGAKARYOCYTIC DIFFERENTIATION**

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**Background.** Telomerase is a ribonucleoprotein reverse transcriptase that adds hexameric repetitive sequences (TTAGGG) to the ends of chromosomes. Telomerase plays a key role in maintaining telomere length and in replicative senescence. Telomerase is active in immature somatic cells and is suppressed in differentiated cells, but the mechanism by which telomerase activity is regulated in relation to cell differentiation remains unclear. Several regulatory mechanisms for telomerase have been identified: (1) transcriptional mechanisms; (2) translational mechanisms, suggesting that the regulation of telomerase activity is a complex process. *Aims. To determine the mechanisms modulating telomerase activity during differentiation in various lineages of hematopoietic cells. Methods. A human chronic myelogenous leukemia cell line (K562) was induced to differentiate into megakaryocytes by exposure to TPA, and into erythroid cells by exposure to STS71. A human acute myeloblastic leukemia cell line (HL60) was induced to differentiate into monocytes by exposure to TPA. To assess the effect of PKC inhibitors during megakaryocytic differentiation, K562 cells were preincubated with Bisindolylmaleimide or Rottlerin, and then TPA was added for further incubation. Telomerase activity, the expression of human telomerase reverse transcriptase (hTERT) protein, mRNA, and functional binding transcription factors within the telomerase promoter region were examined. Cells were separated into cytoplasmic and nuclear fractions to examine the localization of telomerase. Results. TPA induced a transient increase of telomerase activity during the megakaryocytic differentiation of K562 cells, while expression of hTERT decreased gradually throughout differentiation. The transient increase of telomerase was mainly observed in the nuclear fraction rather than the cytoplasmic fraction. Pretreatment of K562 cells with a PKC inhibitor blocked both megakaryocytic differentiation and the transient increase of telomerase activity. Furthermore, a dose-dependent increase of telomerase activity after exposure to recombinant PKC was observed. To further assess the transcriptional control mechanism of telomerase, a chromatin immunoprecipitation (ChIP) assay was performed. STAT3 (which was bound to the hTERT promoter) became dissociated from the promoter during megakaryocytic differentiation, while Sp1 remained stable during differentiation. Conclusions. A transient increase of nuclear telomerase activity was detected during megakaryocytic differentiation stimulated by TPA, and this increase was suppressed by PKC inhibitors. In addition, telomerase activity was dose-dependently increased by recombinant PKC. These results suggest that PKC is one of the post-translational regulators of telomerase activity during the megakaryocytic differentiation of K562 cells. Megakaryocytes are unique hematopoietic cells that undergo DNA replication during differentiation into mature polyploid cells. This may mean that post-translational activation of telomerase is necessary for the immediate stabilization of replicated chromosomes before the de novo synthesis of telomeres commences. On the other hand, STAT3 was suggested to be one of the transcription factors regulating telomerase during megakaryocytic differentiation. These results indicate that telomerase activity during megakaryocytic differentiation stimulated by TPA is regulated at least by two mechanisms, with one being transcriptional and the other being post-translational.

**0331**

**PAX5/TEL CAUSES DOWN MODULATION OF CD19 IN PRE B CELLS**

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Background. We previously cloned the PAX5/TEL chimeric gene, originated from the translocation (9;12)(q11;p13) in an ALL patient. Recent data indicate that the PAX5/TEL fusion defines the cytogenetic entity dic(9;12)(p13;13), a recurrent chromosome abnormality that accounts for about 1% of childhood ALL, almost exclusively B-progenitor ALL. PAX5/TEL is likely to be an aberrant transcription factor, resulting from joining the 3’ region of PAX5 (a transcription factor essential for B cell development) to the 3’ region of TEL/ETV6 (Et’s family DNA binding domain). Aim of the study was to investigate the functions of the PAX5/TEL chimeric protein in preB cells. Methods. We have cloned the FLAG-full length chimeric PAX5/TEL cDNA in the retroviral vector pMSCV-PAX5-TEL-ires-GFP (MigR1). Murine PAX5-/- preB cells and wild type preB cells were transduced with the retroviral construct to analyze cell proliferation, differentiation and growth-dependence on IL-7. Both PAX5-/- preB I cells and wild type preB cells were cultured on OP9 and DL1-OP9 stroma cells. Results. Wild type preB cells, transduced with pMSCV-PAX5-TEL-ires-GFP vector, showed down modulation of CD19 when cultured on OP9 stroma in presence of IL-7. Semi-quantitative RT-PCR didn’t show any difference in transcription of PAX5 target genes such as BLNK, MB-1, M-CSFR. PAX5TEL-preB cells cultured on DL1-OP9 showed a different phenotype, with up-regulation of c-KIT and down-regulation of CD44. PAX5-/- preB cells infected with PAX5TEL and grown on OP9 were CD19 negative even in the presence of PAX5TEL, in absence of IL-7 they died following the same kinetic of the control cells. By semi-quantitative RT-PCR, we didn’t detect mRNAs of CD19 and no difference in BLNK, MB-1 and M-CSFR mRNA level was found. On DL1-OP9, PAX5TEL cells were able to differentiate maintaining the developmental plasticity of PAX5-/- preB I cells. Conclusions. Preliminary results showed a role of PAX5TEL as a transcriptional suppressor, down regulating CD19 expression, thus suggesting a function on B cell differentiation. PAX5TEL cannot replace PAX5 functions in PAX5-/- cells. Further analysis are needed to better evaluate the role of PAX5/TEL protein, both in vivo and in vitro models.

**0332**

**OVEREXPRESSION OF 14-3-3 SIGMA IS ASSOCIATED WITH TYROSINE KINASE ACTIVITY OF P210 BCR-ABL FUSION PROTEIN OF CHRONIC MYELOID LEUKEMIA**

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The 14-3-3 proteins are a family of phosphoserine/threonine-binding molecules and critical mediators of intracellular signaling pathways, including those controlling proliferation, cell cycle checkpoint activation and survival. In particular, upon c-Jun NH2-terminal kinase (JNK)-mediated phosphorylation in response to stress they release c-abl tyrosine kinase and let its nuclear import, the prerequisite for its apoptotic and growth arrest function. Here we show that constitutive tyrosine kinase activity of the p210 bcr-abl fusion protein of chronic myeloid leukemia (CML) is associated with overexpression of 14-3-3 sigma. In 32D cell clones transducing a temperature-sensitive bcr-abl construct the levels of 14-3-3 transcript and protein were increased under permissive culture conditions for p210 TK and significantly reduced by p210 TK inhibition by the TK inhibitor Imatinib mesylate (IM). Moreover, in K562 cell line we observed the hyperexpression of a discrete region of 14-3-3 sigma promoter that corresponds to -8245 to -8508, that was significantly reduced since 4th hour of exposure to IM. Conversely, the methylation status at a CpG-rich area of 14-3-3 sig- ma coding region including the transcription start site (-220 to +116) was not modulated upon p210 TK. Our results support that p210 TK influences 14-3-3 sigma transcription rate by interacting with epigenetic mechanisms that control chromatin accessibility. Interestingly, in K562 cell line IM resistance was associated with a further increase of 14-3-3 sigma expression and higher hyperactivation at its promoter, supporting a putative role of this scaffolding protein in clonal evolution of CML progenitors.
towards drug resistance. Further studies are presently in progress to elucidate the factors that might contribute to heightened expression and enhanced binding properties, whether they may be targeted by drug combinations that have been advanced for clinical trials.

0333
THE INVOLVEMENT OF C18 CERAMIDE AND HUMAN LONGEVITY ASSURANCE GENES IN IMATINIB INDUCED APOPTOSIS
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Background. Ceramides have essential roles in many aspects of cell metabolism, from inflammatory responses through the regulation of cancer-cell growth, cell proliferation, apoptosis, cell migration and senescence. Many cytokines, anticancer drugs and other stress-causing agonists result in increases in endogenous ceramide levels through de novo synthesis and/or the hydrolysis of sphingomyelin. Since human longevity assurance genes (LASS) are responsible for the de novo synthesis of ceramides, the expression levels of LASS genes are important in stress induced apoptosis. Aims. Ceramide metabolism in imatinib induced apoptosis in hematological malignancies was examined in this study. Sensitive and resistant chronic myeloid leukemia (CML) cells, K562 were used as a model system to investigate the changes in ceramide metabolism upon imatinib treatment. Methods. The Ph1 human K562 cells were exposed to step-wise increasing concentrations of imatinib. Subpopulations of cells those were able to grow in the presence of 0.2 and 1 μM imatinib, were then selected, and referred to as K562/IMA-0.2 and K562/IMA1, respectively. Caspase-3 activity was determined using the caspase-3 assay. The mitochondrial membrane potential (MMP) was measured using a JC-1 MMP detection kit. The cellular levels of endogenous ceramides were measured using high performance liquid chromatography/mass spectrometry (LC/MS). Plasmid and siRNA transfection of K562 cells were conducted using an Effectene and DharmaFECT™ siRNA transfection reagent, respectively. Results. Measurement of endogenous ceramide levels by LC/MS showed that treatment with imatinib increased the generation of ceramide, particularly C18-ceramide, significantly in a time-dependent manner in parental sensitive cells, whereas in resistant cells, there was no significant changes in its levels in response to imatinib at 48 hr. Partial inhibition of human longevity assurance gene 1 (hLASS1) by small interfering RNA (siRNA), which is considered as a critical inducer of mitochondrial-mediated apoptosis, significantly reduced imatinib-induced cell death, as detected by activation of pro-caspase-3, and loss of mitochondrial membrane potential, in sensitive K562 cells. In reciprocal experiments, overexpression of hLASS1 caused a marked increase in imatinib-induced C18-ceramide generation and apoptosis in resistant K562/IMA-0.2 and K562/IMA1 cells. Interestingly, analysis of mRNA levels of hLASS1, for the generation of C18-ceramide did not show any significant differences in these resistant cells when compared to controls, suggesting that accumulation and/or metabolism, but not rate of synthesis, might be altered in imatinib-resistant cells. Summary/Conclusions. These data suggest that increased ceramide generation and/or accumulation might be involved in mediating imatinib-induced apoptosis, and that defects in C18-ceramide accumulation and/or metabolism might play a role in a decrease in imatinib-induced apoptosis, thus results in resistance to therapy.

0334
SURVIVIN AND BCL2 EXPRESSION IN CD30-POSITIVE LYMPHOPROLIFERATIVE DISORDERS OF THE SKIN COMPARED TO SYSTEMIC ANAPLASTIC LARGE CELL LYMPHOMAS: AN IMMUNOHISTOCHEMICAL STUDY OF 28 CASES
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Backgrounds. Cutaneous CD30-positive lymphoproliferative disorders (LPDs) are a spectrum of indolent diseases ranging from lymphomatoid papulosis (LyP) to primary cutaneous anaplastic large lymphoma (C-ALCL). Cutaneous anaplastic large cell lymphomas (ALCL), which are potentially aggressive, but has not been elucidated in cutaneous CD30-positive LPDs. Aims. We investigated the expression of two inhibitors of apoptosis, survivin and BCL-2 protein, in a series of cutaneous primitive CD30-positive LPDs and systemic ALCL. Methods. Immunohistochemical analysis was performed with anti-survivin, BCL-2 and Survivin antibodies against ALK1 protein, survivin and BCL-2 protein, on tissue sections with the DAKO Envision system. RT-PCR studies for ALK and ALK/NPM were performed on RNA extracted from paraffin blocks of all 28 cases. Results. All the cutaneous CD30+ LPDs were negative for ALK by immunostaining and RT-PCR. Among systemic ALCL cases, 7 were ALK-negative and 11 were ALK-positive. In the positive cases showed a 366 bp ALK transcript by RT-PCR and the specific NPM/ALK fusion transcript of 98 bp, ruling out the presence of a different rearrangement. All the 28 cases examined showed a clear cytoplasmic positivity for survivin, independently from their clinicopathological group. Five cases of systemic ALCL, which were all ALK negative, showed in addition a nuclear dot-like immunoreactivity for survivin. Nuclear expression of survivin was not observed in the other groups (chi2: p=0.045). Protein BCL-2 cytoplasmic expression was found in 10 cases; systemic ALK-positive ALCL show a lower frequency of BCL-2 expression (chi2: p=0.045). Conclusions. Our result showed that LyP and C-ALCL share a heterogeneous expression of cytoplasmic survivin and BCL-2, similarly to systemic CD30-positive cutaneous tumors. Survivin expression might be expressed also in indolent and potentially regressing lesions and is not an absolute marker of malignancy. Survivin has been indeed demonstrated in many nonneoplastic cells of non-lymphoid nature. BCL-2 was also expressed in half of our cases of LyP and PC-ALCL, similarly to systemic ALCL. Interestingly, both BCL-2 and cytoplasmic survivin expression does not help in distinguishing in the spectrum of cutaneous CD30-positive LPDs nor between cutaneous and systemic diseases. It might be postulated that apoptosis is still potentially inducible in these BCL-2 and survivin-expressing cells because these lesions have the potential to undergo spontaneous regression. Alternative mechanisms including the immune control mediated by activated cytotoxic lymphocytes (CTL) can play a major role in these indolent diseases as postulated in systemic disorders. Our data confirm that BCL-2 is less frequently expressed in ALK-positive than in ALK-negative systemic ALCL cases. The most interesting and unexpected feature was the observation that 45% of our systemic ALK-negative ALCL cases showed nuclear survivin immunostaining, in contrast with others who found survivin exclusively located in the cytoplasm by immunohistochemistry and by Western blotting.

0335
TARGETING IAPs OVERCOMES APOPTOSIS RESISTANCE OF PANCREATIC CARCINOMA CELLS AND SUPPRESSES TUMOR GROWTH AND INVASION IN VIVO
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Pancreatic cancer is one of the leading causes of cancer-related death due to its resistance towards conventional therapies. To improve cancer therapy, it is crucial to better understand the molecular mechanisms underlying apoptosis resistance of pancreatic cancer. Here, we identify X-linked inhibitor of apoptosis (XIAP) as a key determinant of apoptosis resistance of pancreatic carcinoma cells. XIAP was expressed at high levels in the majority of pancreatic carcinoma cell lines and primary tumor samples. Stable downregulation of XIAP by RNA interference significantly reduced viability and enhanced TRAIL-induced apoptosis in pancreatic carcinoma cells. Importantly, knockdown of XIAP also strongly inhibited clonogenicity of pancreatic cancer cells treated with TRAIL, indicating that XIAP promotes clonogenic survival. Stable down-regulation of XIAP significantly increased CD95- and γ irradiation-induced apoptosis, whereas it had no effect on 5-fluorouracil, etoposide or gemcitabine-induced apoptosis. Analysis of apoptosis signaling pathways revealed that knockdown of XIAP resulted in enhanced activation and enzymatic activity of caspase-3, -9, -2 and -8. Furthermore, stable down-regulation of XIAP also led to enhanced drop of mitochondrial membrane potential and increased cytochrome c release after stimulation with TRAIL, indicating that XIAP functions upstream of mitochondria in TRAIL-induced apoptosis. In support of this notion, inhibition of caspase-3 completely inhibited drop of mitochondrial membrane potential in TRAIL-treated pancreatic carcinoma cells. Most importantly, XIAP was knocked down. Most importantly, knockdown of XIAP profoundly inhibited tumor growth and invasion of pancreatic carcinoma cells in vivo. Similarly, inhibition of XIAP by small molecule antagonists sensi-
tized pancreatic cancer cells to TRAIL-, CD95- or γ-irradiation-induced apoptosis. By demonstrating that targeting IAPs significantly enhanced death receptor or γ-irradiation-induced apoptosis and also suppressed tumor growth and invasion of pancreatic carcinoma cells in vivo, our findings indicate that targeting IAPs represents a novel, promising strategy to overcome apoptosis resistance of pancreatic cancer, which has important clinical implications.

0336
TRAIL RECEPTORS IN B-CELL PATIENTS INDEPENDENTLY OF ZAP 70 EXPRESSION
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B-cell lymphocytic leukemia (B-CLL) is an incurable disease with an indolent course characterized by a low growth fraction and a progressive expansion of a subpopulation of monoclonal B lymphocytes (LLC-B cells) which are functionally inactive and resistant to apoptotic cell death. The most specific way to induce apoptosis is stimulation of the different types of death receptors with their specific ligands. TRAIL (Tumour necrosis factor (TNF) -related apoptosis inducing ligand) receptors - a branch of the Fas receptor family, are widely expressed on the surface of many different cell types and induce selective apoptosis of cancer cells but not of normal cells. They can be activated by TRAIL. This ligand can interact with 5 receptors, TRAIL-R1 (DR4), TRAIL-R2 (DR5), TRAIL-R3 (DcR1), TRAIL-R4 (DcR2) e Osteoprotogerin (OPG). The stimulation of the death receptors, DR4 and DR5 can induce apoptosis. However, the expression of DcR1, DcR2 and OPG receptors may counteract TRAIL apoptotic effect. Thus, the expression of these types of receptors can determine the failure to undergo apoptosis in B-CLL and may have direct implications for B-CLL therapy. The aim of this study is to evaluate the expression of TRAIL receptors, DR4, DR5, DcR1 and DcR2 in B Chronic Lymphocytic Leukaemia patients and its correlation with the expression of the prognostic markers ZAP 70 and CD11c. For this purpose directly conjugated monoclonal antibodies to CD5 and CD19 was used to identify LLC-B, T and B cells obtained from 22 patients with B-CLL. These patients were divided according the expression of the ZAP 70 protein (6 ZAP 70+; 6 ZAP 70- and 10 non determined) and the __integer CD11c (10 CD11c+ and 6 CD11c-). The surface expression of TRAIL receptors, DR4, DR5, DcR1 and DcR2, were analysed by flow cytometry, using specific monoclonal antibodies. The results are expressed in Mean Intensity Fluorescence (MIF). We observe in the LLC-B cells lower levels of proapoptotic TRAIL receptors, namely DR5 (18.6±8.1 MIF) compared with normal B cells (35.7 _ 20.5) and higher expression of the antiapoptotic DcR1 receptor which may contribute to resistance of LLC-B cells to death receptor-mediated apoptosis. Preliminary results didn’t show any correlation between TRAIL receptors expression and the above mentioned prognostic markers. However a higher number of patients must be included in the study in order to clarify our results. Acknowledgments. Prof. Catarina Oliveira and Dra Luísa Pais, Directors of Biochemistry Institute, Faculty of Medicine, University of Coimbra, and Histocompatibility Center of Coimbra, respectively

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0337
P38-MEDIATED ACTIVATION OF CASPASES IN C2 CERAMIDE-INDUCED APOPTOSIS OF MOUSE HEMATOPOIETIC CELLS
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Cell-permeable C2-ceramide induces apoptosis in various types of cell. Here, we have studied the effects of C2-ceramide on mouse bone marrow cells containing primitive hematopoietic progenitors (PHPs) with little knowledge about the signaling pathway in the apoptosis induced by C2-ceramide. The C2-ceramide was found to induce apoptosis in a time dependent manner, as defined morphologically by nuclear condensation and fragmentation visualized with propidium iodide staining and by a positive annexin V (AV) and by a reduced colony forming ability of the bone marrow cells of mice. C2-ceramide suppressed colony growth derived from mouse Day-2 post 5-FU marrow cells (5-FU marrow cells) in a dose dependent manner. Incubation of 5-FU marrow cells with 15 µM of C2-ceramide for three hours gave 41.7±17.2% of

mean% control of colonies. Further, an extended study using more PHP- enriched lineage marker-negative cells (Lin- cells), which were isolated from mononuclear 5-FU marrow cells, revealed that C2-ceramide completely suppressed colony formation. To obtain direct evidence of induction of apoptosis in Lin- cells, we detected that about 90% of AV- cells were changed into AV+ cells in Lin- cells by incubation with C2-ceramide, suggesting PHP-mediated cell death specific involvement of caspases in PHP, using the cell-permeable fluorescence-labeled substrates of several caspases and the colorimetric caspase activation assay kits. C2-ceramide treatment showed fluorescence and colorimetric caspases activation of 5-FU marrow cells, which increased in intensity within one hour. However, selective caspase inhibitors inhibited the both fluorescence and colorimetric release of each caspase substrates, indicating the specific involvement of caspases in the C2-ceramide-induced apoptosis of PHP. Further, the selective inhibitors for caspases also prevented nuclear condensation and fragmentation and suppression of colony formation of 5-FU marrow cells. Based on the effect of these caspase inhibitors on the activation of each fluorogenic caspase substrate, C2-ceramide is believed to activate LEHD-, DEVD-, and VEID-cleaving caspases such as caspase- 9, -3, and -6, respectively, in that order. Upstream of the caspases, C2-ceramide activated p38 and the selective p38 inhibitor SB203580, thus reversed the activation of these caspases that had been induced by C2-ceramide, resulting in a significant recovery from apoptosis. Another hand, IETD-cleaving caspase such as caspase-8 was not activated by C2-ceramide. These results suggest that C2-ceramide initiates apoptosis in PHP via activation of the caspase-9-dependent caspase cascade mediated by p38.

0338
CERAMIDE GLYCOSYLATION BY GLUCOSYL CERAMIDE SYNTHASE INHIBITS THE APOPTOTIC EFFECT OF IMATINIB ON HUMAN K562 AND MEG-01 CELLS
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Background: Glucosyl ceramide (Glc-Cer) has recently been shown to be associated with resistance to chemotherapy. Inhibition of glucosyl ceramide glycosylation, whereby pro-apoptotic ceramide is converted to its noncytotoxic Glc-Cer metabolite resulting in drug resistance. Accumulation of Glc-Cer is a characteristic of some MDR cancer cells and tumors derived from patients who are less responsive to chemotherapy. Several tumor cell lines and clinical samples have been shown to overexpress Glc-Cer synthase (GCS) enzymes that transfer glucose from UDP-glucose to ceramide and produces Glu-Cer. Aims. The effects of GCS in imatinib resistance in human chronic myeloid leukemia (CML) cells were investigated. The cells were exposed to a combination of imatinib and GCS inhibitors, D-threo-1-phenyl-2-decanoylamino-3-morpholino-propan-1-ol (PDMP) or N-(α-Nonyl)hydroxygalactonojirimycin (C9DGJ) to determine if resistant cells could be sensitized. Methods. The Ph+ human K562 and Meg-01 cells were exposed to step-wise increasing concentrations of imatinib. Subpopulations of cells that were able to grow in the presence of 0.2 and 1 µM imatinib, were then selected, and referred to as K562/ or Meg-01/IMA-0.2 and Meg-01/IMA'1, respectively. Flasmid transfection of K562 cells were conducted using an Effective transfection reagent. The expression pattern of GCS was detected by RT-PCR and Western blotting. The IC50 values were determined from cell survival plots obtained by MTT. Cell cycle profiles of cells were analyzed by flow cytometry. The cellular levels of endogenous ceramides were measured using high performance liquid chromatography/mass spectrometry (LC/MS). Results. Measurement of the levels of GCS by RT-PCR and Western blotting demonstrated that the expression of GCS was increased in both K562/IMA-1 and Meg-01/IMA-1 cells as compared to parental sensitive cells. The possible role of GCS in resistance to imatinib was further examined by transfection and the overexpression of GCS gene in K562 and Meg-01 cells. GCS overexpression in sensitive cell lines resulted in an increase in imatinib-resistance. There was a significant increase of apoptosis by co-application of imatinib and GCS inhibitors (PDMP or C9DGJ) after 48 hr in both sensitive and resistant cells. There was also an up-regulation of ceramide levels measured using high performance liquid chromatography/mass spectrometry (LC/MS) by co-application of imatinib and GCS inhibitors (PDMP or C9DGJ) to K562 and Meg-01 cells. Summary/Conclusions. These results may suggest the involvement of GCS, by converting pro-apoptotic ceramide to Glu-Cer, in imatinib resistance. Besides, inhibiting the GCS activity by co-appli-
cation of PDMP and/or C9DGJ increased the sensitivity of CML cells to imatinib.

0339 NOSCPAPNE INDUCES APOPTOSIS THROUGH ACTIVATION OF CASPASES AND MITOCHONDRIAL EVENTS IN PS3-NULL MYELOBLASTIC LEUKEMIA CELL LINE K562

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Monitoring apoptosis is becoming increasingly important in finding new chemotherapeutic drug and their mechanism. Previously, the microtubule opium alkaloid noscapine was discovered as a microtubule destabilizing agent that arrests mammalian cells at mitosis and induces apoptosis. Because noscapine is water-soluble and absorbed after oral administration, and has little toxicity to normal tissue and no inhibition of immune responses, its chemotherapeutic potential in human cancer merits thorough evaluation. We selected drug resistant, PS3-null myelogenous leukemia cells to monitor apoptosis and study of noscapine’s mechanism. K562 cells showed delayed but effective response to noscapine treatment, and we could monitor apoptosis by the DNA fragmentation, PARP cleavage and increasing activity of caspase 2,3,6,9 with 20 µM noscapine after 24-48hr treatment. The increased Bax/Bcl-2 ratio more than three times with 20 µM noscapine in time dependent manner from 3-48 hr can prove some mitochondrial event in response to this drug. These results help to elucidate some critical points in noscapine mechanism as a good candidate for preventive and therapeutic application in chronic myeloid leukemia.

0340 ERYTHROID-SPECIFIC TRANSCRIPTIONAL REGULATION OF THE HUMAN PROTOPHORYMIN OXIDASE GENE IS MEDIATED BY TWO GATA-1 SITES IN EXON 1

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Background. Protoporphyrinogen oxidase (PPOX) catalyzes the six-electron oxidation of protoporphyrin IX to protoporphyrin IX. Like other heme biosynthetic proteins, PPOX is involved in synthesizing heme for red cells (erythroid-specific expression) and heme as a cofactor for the respiratory cytochromes (housekeeping expression). Where as tissue specific regulation of other heme biosynthetic enzymes is extensively studied, there is little knowledge concerning transcriptional regulation of PPOX. Aims. The aim of this study was to investigate molecular mechanisms involved in the erythroid-specific regulation of PPOX. Methods. Functional studies were performed using transient transfection of PPOX promoter constructs in human K562 erythroleukemia cells. DNA-protein interaction at the GATA-1 sites in exon 1 of PPOX was studied using Electrophoretic Mobility Shift Assay’s (EMSA) with nuclear extracts from K562 cells. Results. In vitro transfections studies revealed that reporter constructs containing exon 1 showed a 300% increase in promoter activity compared to constructs lacking this exon. Transfection experiments of wild-type and mutant reporter plasmids in K562 cells demonstrated that erythroid-specific transcriptional regulation of PPOX was mediated by two GATA-1 sites in exon 1. The highest level of transcription depended on the integrity of both sites. Electrophoretic mobility shift assay and supershift experiments using K562 nuclear extracts demonstrated that both GATA sites were able to bind GATA-1 in vitro. Exon 1 did not have any effect on PPOX promoter activity in human hepatoma HepG2 cells. In HeLa human cervical carcinoma cells, however, the presence of exon 1 decreased promoter activity. Summary/Conclusions. Exon 1 of the human PPOX gene contains two GATA-1 binding motifs, which both are required for erythroid-specific expression of PPOX and, in addition, bind GATA-1 in vitro. These results contribute to a better understanding of the molecular mechanisms involved in differential regulation of the human PPOX promoter in erythroid and non-erythroid cells.
CLINICAL AND PROGNOSTIC SIGNIFICANCE OF P53 GENE MUTATION IN ACUTE LEUKEMIA

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Background and aim of the work. P53 is a tumor suppressor gene, located at the chromosomal region 17p13, consisting of 10 introns and 11 exons, of which exons 2 to 11 are transcribed. Wild-type (wt) P53 acts as a tumor suppressor protein whereas mutant (mut) P53 may exhibit gain of function properties such as immortalization of primary tumor cells. The P53 protein plays a crucial role in maintaining genetic stability at the cellular level. This work was planned aiming to recognize the potential role of P53 in leukemogenesis and explain the correlation between mutations of P53 in acute leukemia patients and clinical subtypes, clinical behavior and prognosis of the disease. Subject and methods. This study was carried on 38 patients with acute leukemia. Twenty-eight of them were newly diagnosed patients, and ten patients were in relapse. Accordingly they were categorized into 3 groups: Group I (at diagnosis): This group was included 28 newly diagnosed patients with acute leukemia. They were classified into 15 cases with ALL and 13 cases with AML. Group II (after induction): this group was included 28 patients who were followed up after induction for 1, 6, and 12 months. Group III: This group included 10 relapsed patients with acute leukemia (sampling taken once at relapse). They were classified into 5 cases with ALL and 5 cases with AML. In addition 10 subjects were selected as control group. Patients and controls were subjected to the following laboratory investigation: Complete blood picture, erythrocyte sedimentation rate, Liver function tests and serum creatinine, uric acid and serum LDH. Bone marrow aspirate, cytochemistry stains on blood or bone marrow smears: were helpful in distinguishing AML from ALL and in subclassifying AML. Assay of mutant p53 protein by immunophenotyping techniques. Detection of p53 gene mutation by PCR-SSCP and Sequencing techniques (ABI 310 genetic analyzer, Perkin Elmer). Assay of mutant p53 protein using FITC-conjugated monoclonal mouse anti-human p53 protein clone DO-7 Code No.7054 Lot 050. Edition 15.06.00. Results. In this study we found that, the incidence of p53 mutations were higher in ALL patients (13.3%) than AML patients (7.6%) cases who showed p53 mutations at diagnosis were among cases who resisted chemotherapy (3 cases out of 8), they had exon 5 and 6 mutations. P53 mutations showed higher incidence in relapsed AML and ALL patients (20% and 60% respectively). Exon 8 mutations were the most frequent type of mutation, affecting mainly relapsed AML and ALL. Followed by exon 6 and exon 7 mutations, that were restricted to relapsed ALL cases. Summary and conclusions. P53 mutations were prevalent infrequently in de novo acute. Leukemia higher incidence of p53 mutations were in aggressive and relapsed leukemia. ALL was associated with higher incidence of p53 mutations than AML. The p53 mutations-bearing patients did not differ in their clinical and laboratory data from those without mutations. Mutations of exon 8 were found in high frequency in relapsed acute leukemia (ALL and AML). Mutations of exon 5 were found in acute leukemia patients with poor response to chemotherapy. Mutations of exon 6 were found in patients with poor response to chemotherapy and in relapsed ALL. Mutations of exon 7 were found in relapsed ALL. Mutations of p53 usually affect both alleles with positive (LOH).
published results. Results. Acute lethal X-GVHD generally occurs with in 3 weeks of the transfer of fresh huPBMC (15x106 CD3- cells) into RAG2-/-γc-/- mice. X-GVHD occurred in 99% of 68 mice, including 87% acute lethal X-GVHD and 12% chronic X-GVHD. For 14 different donors, no significant difference between donors was observed with regard to development of acute X-GVHD. Acute X-GVHD could be effectively prevented by FK506 sc at day 0 resulting in 100% survival of mice, although FK506 administered later time points within treatment with prednisolone iv or OKT3 sc did not abrogate acute X-GVHD nor did IL-2 ip exacerbate acute X-GVHD. Further analysis showed the overall impact of ex vivo culture on development of X-GVHD. There was a significant linear correlation for fresh huPBMC in dose-response of 66 mice (r2 = 0.58, p<0.000) and for cultured huPBMC in 130 mice (r2 = 0.8, p<0.001). In contrast to fresh huPBMC, only 44% of mice developed X-GVHD after injection of huPBMC (15x106 CD3+ cells) that were cultured and stimulated with OKT3, including 25% acute lethal X-GVHD and 19% chronic X-GVHD. Strikingly, this was different for CD83/28 costimulated huPBMC, of which 58% developed X-GVHD, including only 10% acute lethal X-GVHD and 48% chronic X-GVHD. These results suggest that CD83/28 costimulation of huPBMC stimulates the development of chronic X-GVHD. We speculate that a more efficient activation by CD28-ligation leads to an increase in in vivo survival and proliferation of human T cells that permits the development of chronic X-GVHD. Conclusion. The huPBMC-RAG2-/-γc-/- xenogeneic transplant model can be considered a useful sensitive model to date for evaluation of human T cells in vivo and will be a valuable addition to current allogeneic murine T cell models. Future studies will involve further exploration of the influence of CD83/28 costimulation on development of chronic X-GVHD.

CD4+CD25+ REGULATORY T CELLS ARE GENERATED AFTER BONE MARROW TRANSPLANTATION WITH REDUCED INTENSITY CONDITIONING REGIMENS IN AN ANTIGEN SPECIFIC FASHION

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CD4+CD25+ regulatory T cells are generated after bone marrow transplantation with reduced intensity conditioning regimens in an antigen specific fashion. Background. It has been shown that graft-versus-tumour (GvT) effect plays a prominent role in eradicating the underlying malignancy after allogeneic bone marrow transplantation (BMT) for leukemia patients. The therapeutic GvT effect relies on the establishment of mixed haematopoietic chimerism which produces host-versus-graft (HvG) tolerance and allows donor lymphocyte to be infused and expanded. Reduced intensity conditioning regimens have been developed to assist the establishment of HvG tolerance in patients and animal models. Aims. To investigate the mechanisms of HvG tolerance after RIC-BMT. Methods. Female C57BL/6 mice were transplanted with male bone marrow cells after sublethal total body irradiation (400cGy). Under these conditions, male donor cell engraftment was achieved and HvG tolerance established within 4 weeks after BMT. FACS analysis was carried out to number CD4+CD25+ and CD4+FoxP3+ cells in recipient mice. Unfractionated splenocytes or CD4/CD25 depleted cells from chimeric mice were added to MLRs to test their suppressive ability in vitro on the proliferation of male-specific T cells in response to HY as measured by H-2b-Hy-antigen tetramer staining. The immunosuppressive effect of unfractionated or CD4/CD25 depleted chimeric splenocytes was also tested in vivo using CFSE-labelled male/female cell mixture as targets. The effect of chimeric splenocytes was also assessed for their ability to favour engraftment of CD4+FoxP3+ cells from the peripheral blood of healthy volunteers and patients with CML by tetramer staining, multi-color flow cytometry and enzyme linked absorbent spot (ELISPOT) assays. Results. In the current study we have analyzed the effect of bortezomib in both DCs' viability and maturation as well as the ability of bortezomib-treated DCs to generate a tolerogenic T-cell response. Results. Bortezomib showed a detrimental effect on DCs viability at 50nM and it was specially evident in cases cultured in the presence of TNFa and LPS while cell viability was not significantly affected in cultures performed without TNFa and LPS, indicating that bortezomib induced apoptosis of DCs in conditions which induce fully DCs activation. We next tested the effect of bortezomib on the expression of co stimulatory molecules and on the cytokine pattern of DCs. Interestingly, the addition of bortezomib decreased the expression of CD86 both in un-stimulated or fully activated (TNFa and LPS treated) DCs while the expression of CD80, CD40 and HLA-DR was also modified at 10nM of the drug. Concerning the cytokine pattern, the intracellular expression of IL-12 significantly decreased at a concentration of 10nM of the drug. In addition, we evaluated the effect of the DCs
cured with or without bortezomib at 10 nM in the activation pattern and cytokine profile of T-cells after mixed lymphocyte cultures (MLRs) and we found that, among MLRs performed using un-stimulated and/or stimulated with the drug, 20% (95% CI = 5.5–81) of T lymphocytes were activated as compared to 43% (95% CI = 11.5–88) among T cells co-cultured with DCs fully activated with TNFα and LPS (p = 0.02), by contrast to activated DCs were 21% (95% CI = 2.8–0.46) vs 31% (95% CI = 16–48) (p = 0.04) when they were co-cultured with bortezomib-treated CD54 naïve with fully activated with TNFα and LPS, respectively. This results indicated that bortezomib-treated DCs were unable to properly stimulate T cells even after exposure to a proinflammatory milieu. Moreover, T lymphocytes previously cultured with bortezomib-treated DCs were unable to become activated when they were further stimulated with fully activated untreated DCs from the same donor, indicating that T lymphocytes exposed to bortezomib-treated DCs become tolerant to the antigens presented by DCs. Conclusion. Bortezomib changes viability and modifies the maturation and cytokine pattern of DCs. These latter effect results in an impaired capability to induce allogeneic T cell stimulation and generates a tolerogenic response of T cells cultured with bortezomib-treated DCs. These results suggest a potential role for the in vivo use of the drug prior to allogeneic transplantation through its effect on host DCs and/or for the in vitro generation of tolerogenic DCs.

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STEM CELL FACTOR ENHANCES T-CELL RECOVERY AND THYMOPYEOISIS FOLLOWING EXPERIMENTAL BMT

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Deficient thymopoiesis and retarded recovery of newly developed naïve CD4+ T-cells is one of the most important rate-limiting steps in the development of impaired immune competence following hematopoietic stem cell transplantation (HSCT). Recently, we showed that Fls-like tyrosine kinase-3 ligand 3 (FL) accelerates T-cell recovery following experimental bone marrow transplantation (BMT) via expansion of bone marrow (BM) lymphoid progenitors prior to recovery of thymopoiesis. Several studies have suggested an important role for stem cell factor (SCF)-c-kit interactions in T-cell development, but is unclear at which level SCF primarily affects T-cell development. Here we evaluated whether SCF would affect T-cell recovery, thymopoiesis and BM lymphoid progenitor cell numbers following experimental BMT. Three Gy irradiated Rag-1−/− mice (C57Bl/6 Ly5.2 background) were used as recipients of T-cell depleted congenic bone marrow cells (4 × 105 x C57Bl/6 Ly5.1 origin). Mice were treated with PBS, SCF (100 µg/kg s.c.), FL (800 µg/kg) or SCF combined with FL 3 times weekly for 4 weeks following BMT (n = 6/group). Peripheral blood (PB), splenic and thymic lymphocyte subsets and BM lymphoid progenitors (LSKflt5−, LSKflt5+, common lymphoid progenitors (CLP) or common myeloid progenitors (CM) using FACS analysis at day 28 post-BMT. SCF or FL-treated mice showed higher numbers of both PB and splenic T-cells as compared to PBS-treated control mice (PBS vs. SCF [mean absolute splenic T-cell numbers ×10^6 /splenocyte±SEM]: 0.3±0.1 vs 4.5±2.2; p = 0.02). No additive or synergistic effect was observed in mice treated with both SCF and FL. In contrast to FL, SCF did not increase peripheral B-, NK and dendritic cell numbers. SCF- or FL-treated mice showed an increase in thymic cellularity (PBS vs. SCF [mean absolute cell numbers ×10^6 /thymus ±SEM]: 6.2±0.2 vs. 11.4±1.1; p = 0.46), numbers of donor-derived thymocytes (0.13± 0.08 x 10^6 vs. 7.2± 4.5; p = 0.06) and numbers of all thymocyte subsets, including DN (0.5± 0.1 x106 vs. 1.6± 0.4; p = 0.07), DP (10± 7 vs. 47.0± 56; p = 0.1), CD8SP (1.8± 0.7 vs. 16± 8; p = 0.008) and CD8SP (0.8± 0.1 vs. 6± 0.3; p = 0.04). In a trend toward increased percentages and absolute numbers of BM LSKflt5− was observed in SCF-treated mice (PBS vs. SCF [mean absolute BM LSKflt5− cell numbers ×10^6/feumur ±SEM]: 8±4 vs. 5±24; p = 0.12). These data show that SCF may enhance T-cell recovery by improvement of thymopoiesis and possibly also by expansion of lymphoid progenitors after BMT. These results may provide a rationale for clinical application in recipients of HSCT with a retarded T-cell recovery mainly due to transplantation of limited numbers of progenitor cells such as may occur in cord blood transplantation.

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IS BODY-WEIGHT-BASED CALCULATION OF IV BUSULFAN FIXED DOSE THE APPROPRIATE DOSE OF BUSULFAN IN CHILDREN UNDERGOING HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background. High-dose oral busulfan (Bu) is often included in conditioning/preparative regimens prior to autologous (auto) or allogeneic (allo) transplantation (T). Studies have reported that pharmacokinetics (PKs) of Bu in HSCT are age-dependent with underexposure of children received the usual dosage 16 mg/kg over 4 days. Bu clearance (CL) is highly variable in children and increased in the youngest. Thus age-based dosing and therapeutic drug monitoring (TDM) with dose adjustment is needed to target an area-under-the-curve-plasma-concentrations (AUC) equivalent to adults. An IVBu form was developed. In a first study (US trial), 24 children received an IVBu age-based dosing with TDM equivalent to the oral (1.0 mg/kg ≤ 4y and 0.8 mg/kg > 4y) (Wall D., ASH 2006, #2066). A retrospective analysis suggested that the dose of Bu significantly must be calculated on the basis of the body-weight (BW) (Nguyen L et al BMT 2004). To validate the new dose-regimen a prospective study was conducted. Aims. To prospectively validate a new body-weight-based fixed dose of IVBu in children in its ability to target an AUC within a predefined therapeutic window for more than 75% of patients without any therapeutic drug monitoring. Methods. PKs of IVBU administered at the defined dosage were studied in children who received either IVBu/Melphalan or IVBu/Cyclophosphamide prior to auto- or allo-T, respectively. IV Bu (16 doses) were administered over 2 h at 1.0 mg/kg, 1.2 mg/kg, 1.1 mg/kg, 0.95 mg/kg, and 0.8 mg/kg for patients (pts) with <9 kg, 9 to <16 kg, 16-23 kg, >23-34 kg, and >34 kg strata of body weight, respectively. PK was performed at doses 1, 9 and 13 but no dose adjustment was allowed. Bayesian Bu AUCs were calculated. Results. Preliminary results are available in 55 pts, median age 6y (0.3-17.2), including 20 pts ≤ 4 y. A significantly better AUC targeting (900-1550 µM.min) was achieved with the new fixed dose as compared to the usual age-based dosing (76% vs. 54%; p = 0.001). Moreover, there is no longer a significant difference in systemic exposure (mean±s.d.: 124±205 µM.min) between children treated with this new dosage and adults given 12.8 mg/kg of IVBu, although significant differences (p = 0.001) on Bu CI were observed among weight groups. AUCs were highly variable in children (>2 fold). Conclusion. A highly variable in children (>2 fold).}
of human platelets in mouse blood is $1 \times 10^9$/ml and that the injection of 0.5 μg (human) Tpo right after transplantation of unmanipulated MPB stem cells does not affect the numbers of human blood platelets or the percentage of human hematopoietic cells in the mouse bone marrow (BM). Next, MPB CD34+ cells were cultured for 7 days in the presence of Tpo (100 ng/ml) and IL-1B (10 ng/ml). An expansion of approximately 6-fold was observed after 7 days of culture. Over 50% of the expanded cells expressed CD41, but the numbers of CD34 expressing cells were detected. After sublethal irradiation, NOD/SCID mice were transplanted with unmanipulated CD34+ cells (group A), unmanipulated cells combined with ex vivo generated MKs (group B, C, D), or ex vivo generated MKs only (group E; see Table 1 for the dosing scheme). As control, the mice of group F did not receive any cells after irradiation. Blood was collected at day 3, 7, 10, 14, 21 and 28 after transplantation. Already after three days human platelets could be detected in the blood of the mice that received the highest number of cultured cells (group C and E). After 7 days, human platelets were detected in the blood of the mice from all groups, except the mice of group A, which received only uncultured cells. In the mice of the groups A, B, C and D platelet numbers increased till day 14 (to an average of $6.9 \times 10^9$/ml blood) with a small decrease towards day 21 ($5.9 \times 10^9$/ml) and day 28 ($4.5 \times 10^9$/ml). The mice of group E reached a maximum of $3.4 \times 10^9$ human platelets per ml blood at day 10 and numbers declined from thereon. At day 21 human platelets in the mice of group E were barely detectable. The experiment will be terminated at day 55 and chimerism will be determined in the blood, BM and spleen. In summary, expanded MKs can significantly contribute to thrombopoiesis during the first days after transplantation. This indicates that the period of thrombocytopenia after intensive chemotherapy can be overcome by the co-transplantation of ex vivo expanded CD34+ MKs. Since previously published clinical trials showed only a small effect of co-transplanted MKs it may be interesting to extend our protocol to a clinical setting.

### Table 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>Cells</th>
<th>Cells transplanted</th>
<th>Mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>MPB CD34</td>
<td>4.5x10^6 NC/mouse</td>
<td>100% 4</td>
</tr>
<tr>
<td>B</td>
<td>MPB CD34 + expanded CD34</td>
<td>4.05x10^6 NC + 0.45x10^6 NC input culture/mouse</td>
<td>90%-10% 4</td>
</tr>
<tr>
<td>C</td>
<td>MPB CD34 + expanded CD34</td>
<td>4.5x10^6 NC + 4.5x10^6 NC input culture/mouse</td>
<td>100% 100% 4</td>
</tr>
<tr>
<td>D</td>
<td>MPB CD34 + expanded CD34</td>
<td>2.25x10^6 NC + 2.25x10^6 NC input culture/mouse</td>
<td>50%-50% 4</td>
</tr>
</tbody>
</table>

### 0351

**THE T CELL RECEPTOR REPertoire USAGE DIFFERS BETWEEN CD4+CD25+ REGULATORY T CELLS AND THEIR CD4+CD25- COUNTERPART AFTER ALLOGENIC STEM CELL TRANSPLANTATION**

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**Background.** After allogeneic haematopoietic stem cell transplantation (SCT) the overall T cell receptor (TCR) repertoire is characterized by a lower diversity and a markedly skewed pattern. Its normalization may contribute to thrombopoiesis during the first days after transplantation. This indicates that the period of thrombocytopenia after intensive chemotherapy can be overcome by the co-transplantation of ex vivo expanded CD34+ MKs. Since previously published clinical trials showed only a small effect of co-transplanted MKs it may be interesting to extend our protocol to a clinical setting.

Through capillary electrophoresis. Conventional spectratory evaluation was carried out by calculating an overall complexity score and by determining the percentage of skewed and oligoclonal Vβ profiles. Moreover, we developed a new analysis method to quantify the proportion of Vβ subfamilies with similar profile between the Treg subset and its Tconv counterpart. Results. Although we observed a significantly higher percentage of skewed and oligoclonal Vβ subfamilies in both cell subpopulations less than 1 year after SCT, the high-dimensional analysis systems showed essentially similar TCR patterns between Treg and Tconv cells. We then compared the spectratory profiles of the 2 cell subsets within each Vβ subfamily in each subject. As a tool we developed a new "similarity score" expressing the proportion of Vβ subfamilies with similar profile between Treg and Tconv subpopulations. We detected a positive correlation between similarity score and time after SCT (Pearson correlation coefficient = 0.65). A higher score was observed in patients more than 3 years after allografting (mean 0.90 vs. 0.61, p = 0.01). Noticeably, in patients less than 3 years after SCT the differences were very often ascribable to the detection in the same Vβ subfamily of an oligoclonal profile in the Tconv but not in the Treg subpopulation. This specific pattern was almost exclusively confined to this group of patients (mean 52% vs. 5%, p = 0.002).

### 0352

**THE GRAFT VERSUS HOST DISEASE AND SURVIVAL MIGHT BE DIFFERENT ACCORDING TO ADMINISTRATION ROUTE AND Dose OF MeseCHNIAL MURINE HEMATOPOIETIC STEM CELL TRANSPLANTATION**

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**Background.** The state engraftment and graft versus host disease (GVHD) should be overcome in allogeneic hematopoietic stem cell transplantation (HSCT). Mesenchymal stem cells (MSC) contributed to sustain early engraftment and lessen GVHD. In conventional HSCT, a lot of cells were reconstituted into liver or lung during intravenous infusion. Aims. We evaluated whether survival and GVHD in HSCT would be different according to administration route, and dose of MSC. Methods. We retrieved MSC through 5 consecutive subculture of C3H/10T1/2. All lethally irradiated 6 weeks-old female Balb/c mice received 1x10^5 human platelets together with 1x10^5 bone marrow cells and 5x10^6 spleen cells of female C3H mice according to route and dose. The study groups were divided into the intravenous (IV) and intra-marrow injection (IBM) according to route, and also dose of MSC. In co-administration of MSC, mice were designed in HSCT with 1x10^5 MSC and 1x10^4 MSC, and some mice received with addition of MSC on post-HSCT 48 hours. All mice were observed daily for survival and GVHD clinical status. Results. All mice without MSC died with no different GVHD pattern in post-HSCT 8 day in spite of route. In HSCT with MSC, there were no difference of survival rate and GVHD score in mice co-transplantation with 1x10^5 MSC, and also with addition of MSC on post-HSCT 2 day in both IV and IBM group. However, mice received with 1x10^5 MSC were significantly better survival and lower GVHD score than others in both groups, although mice in IV group were longer survival than in IBM group. Conclusions. Our data suggested that the administration route of cells would not affect survival and GVHD pattern, and co-transplantation with high dose of MSC might prevent lethal GVHD in MSC mismatched allogeneic murine HSCT. We concluded that HSCT with IV infusion of high dose of MSC might prevent lethal GVHD and have survival benefit.

### 0353

**TWO ISOFORMS OF HUMAN FOXP3 POSSESS SIMILAR CAPACITIES TO INDUCE DIFFERENTIATION OF REGULATORY T CELLS FROM CD4+CD25- T CELLS**

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University Medical Center Utrecht, UTRECHT, Netherlands

**Background.** Naturally occurring CD4+CD25+ regulatory T cells (Tregs) are considered to play important roles in the clinical outcome of stem cell transplantation (SCT). The forkhead/winged helix transcription factor, Foxp3 is the key factor for the differentiation of Tregs. While in rodents the Foxp3 gene is expressed as a single transcript, in humans it...
Treg cell numbers with the clinical outcome of SCT will benefit from
lympho(1G. Vassal et al.)-transduced CD4+CD25- Foxp3- cells. Foxp3 transduced T cells were cultured briefly for 95% purity before phenotypical and functional characterization. Results. In PBMC of healthy individuals, both Foxp3 isoforms were preferentially expressed in CD4+CD25high cells. However, there was no quantitative relation between the gene expression levels of these isoforms. In Treg clones, generated by limiting dilution of CD4+CD25hi cells Foxp3 isoforms were expressed simultaneously; but there was no quantitative correlation between their expression levels. Phenotypic and functional analyses of Foxp3 transduced T cells revealed that T cells transduced either with Foxp3FL or with Foxp3'E2 genes expressed high levels of CD25, CTLA-4 and GTR; were anergic to stimula-
tion via CD3 and subsequently the CD3 induced proliferation of autol-
ogous and allogeneic CD4+CD25- cells in a dose dependent manner. Summary and Conclusions. Our results reveal that the two isoforms of human FOXP3 possess similar capacities to induce differentiation of Tregs from CD4+CD25- T Cells. Since they can be quantitatively expressed independently from each other, studies aiming at corre-
gating the clinical outcome of SCT will benefit from quantiative determination of both Foxp3 isoforms.

**0354**

**BODY-WEIGHT-BASED IV BUSULFAN FIXED DOSING AS PART OF BUMEL REGIMEN BEFORE AUTOLOGOUS TRANSPLANTATION IN CHILDREN WITH HIGH RISK SOLID TUMORS: REDUCED TOXICITIES IN THE FRENCH PROSPECTIVE MULTICENTRE STUDY**

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1Institut Gustave Roussy; VILLEJUIF, France; 2Hôpital La Timone, MARSEILLE, France; 3Institut Curie, PARIS, France; 4Centre Leon Berard, LYON, France; 5Hôpital Dieu, CLERMONT-FERRAND, France; 6Institut de Recherche Pierre Fabre, BOULOGNE BILLANCOURT, France

Introduction. Oral busulfan (Bu) and melphalan (Mel) has been exten-
sively used as a high-dose chemotherapy regimen followed by hematopoietic stem cell transplantation (HSCT) in pediatric patients (pts) with high-risk solid tumors. Bu has a narrow therapeutic window. With the availability of IV Bu, a new dosing strategy based on body weight (BW) has been defined allowing to target area under the curve (AUC) without any therapeutic drug monitoring (TDM) (Nguyen L. et al. BMT 2004). We assessed prospectively the new IV Bu body-weight-
based dosing. The Pharmacokinetics results and hematological results are reported separately (G. Vassal et al.), we report here the clinical outcomes of autologous pts. Aims. To investigate the safety of this new IV Bu dosing strategy, to assess hematopoietic recovery, and to evaluate the consequences of IVBu dosage upon children clinical outcome. Patients and Methods. Twenty-seven children (14 male/13 female) received IV Bu over 2 h at a dose of 1.0 mg/kg, 1.2 mg/kg, 1.1 mg/kg, 0.95 mg/kg, and 0.8 mg/kg for pts with <9 kg, 9-<16 kg, 16-23 kg, 23-34 kg, and >34 kg strata of weight, respectively. Mel 140 mg/m² was then administered followed by HSCT. Clon-
azeapam was given as seizures prophylaxis. Indications for HSCT were: high risk neuroblastoma [NB; n = 24]. CR1/CR2, 10 VGFR, 5 PR1/PR2), Ewing Sarcoma [EW, n = 3, 1 CR1, 2 PR2], Med. agn, 6 PR1, 3A.O. San Giovanni Battista, TORINO, Italy; 3Istituto Nazionale Tumori, MILAN, Italy; 4A.O. San Giovanni Battista, TORINO, Italy

**0356**

**PERMANENT TELOMERE LOSS IN MYELOCID CELLS FROM LONG-TERM SURVIVORS OF LYMPHOMA PATIENTS TREATED WITH HIGH DOSE CHEMOTHERAPY AND AUTOGRAFT**

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1University of Turin, TORINO, Italy; 2Istituto Nazionale Tumori, MILAN, Italy; 3A.O. San Giovanni Battista, TORINO, Italy

Background. Telomere length (TL) decreases at each cell division and therefore it is a good marker of cell replication history. Accelerated cell proliferation is required during post-autograft hematopoietic recovery
resulting in abnormal telomere loss at least in the early period after autologous bone marrow transplantation. Aims. To evaluate both TL and committed progenitors is also maintained at long-term in spite of the period following autograft; iii. a marked reduction of BM immature and committed progenitors is found to be markedly reduced compared to normal controls (data not shown). Again, no correlation between degree of progenitor reduction and time-interval since autograft was observed. Conclusions. i. telomere length is reduced in myeloid cells from subjects surviving up to 10 years following autograft; ii. TL reduction is long-lasting suggesting that telomere-elongating enzymes are unable to reconstitute a normal telomere length even after a prolonged period following autograft; iii. a marked reduction of BM immature and committed progenitors is also maintained at long-term in spite of the large amounts of transplanted CD34+ cells. Thus high-dose chemotherapy and PBSC autograft may result in myelopoietic cell abnormalities that appear to be irreversible. This observation is of both biological and clinical relevance.
AM3 THERAPY PREVENTS MUCOSITIS IN PATIENTS UNDERGOING HEMATOPOIETIC STEM CELLS TRANSPANTATION

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Introduction. AM3 (Inmuneferon™), is a glycoconjugate of natural origin with immunomodulatory properties indicated in secondary immunodeficiencies as well as coadjuvant treatment in neoplastic diseases with cellular immunity deficiency. Aim. To evaluate the influence of AM3 in the biological recovery of immune system in patients undergoing hematopoietic stem cells transplantation (HSCT) (autologous/allogenic).

As well as to register the clinical incidences as undercurrent infections and mucositis from day of infusion hematopoietic cells to 90 days post-transplantation in patients with and without AM3. Patients and Methods. Group A (cases): Inclusion of 19 consecutive patients undergoing HSCT from February 2003- March 2004. Biological evaluation of the immune system recovery: BCC, immunomarkers quantification: IgG, IgA, IgM, C3, C4, lymphoid populations distribution and activity of monocyte biomarkers (chitotriosidase, CCL18/PARC) on days 7, 0, +7, +14,+21,+28,+56,+90. Clinical evaluation and comparing cases/controls of incidence of mucositis severity evaluated according to WHO classification and microbiological documented infectious diseases. Results. The addition of AM3 as a coadjuvant therapy in patients undergoing HSCT reduces significantly the percentage of oral mucositis (61.5% vs 89.5%) (p=0.025) being those mucosits of less complexity in the 2 first weeks posttransplantation. The number of infectious diseases was lower in patients under AM3 therapy (31.5% vs 42%) (p=0.254) (Detailed in table). The recovery of the immune system evaluated by blood cells count and immunomarkers showed a initial recuperation at day +14 in autologous HSCT and at day 21 for allogenic HSCT. Tolerance was satisfactory and only two patients needed discontinuation therapy because of digestive intolerance. Wide studies must be performed in order to evaluate the benefit of this coadjuvant therapy. This work has been partially sponsored by a grant from FEHHA.

Table 1.

<table>
<thead>
<tr>
<th>Mean age (range)</th>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females</td>
<td>6 (31%)</td>
<td>7 (36,8%)</td>
</tr>
<tr>
<td>Alllogenic HSCT (M/F)</td>
<td>4 (21%) / 5 (19,1%)</td>
<td>6 (31.5%) / 5 (19,2%)</td>
</tr>
<tr>
<td>Autologous HSCT (M/F)</td>
<td>15 (78,9%) / 5 (12%)</td>
<td>13 (68,4%) / 5 (12,5%)</td>
</tr>
</tbody>
</table>

Diagnosis

| Acute leukaemia: 7 (37%), MM 7 (37%), MDS 2 (10%), Hodgkin disease 2 grade II, lymphoblastic lymphoma 1 (5%) | Acute leukaemia: 4 (21%), MM 9 (47%), Hodgkin, disease 2 grade II, lymphoblastic lymphoma 1 (5%) |

Mucositis

| 12 (63%); grade I: 1 patient (9%) grade II: 4(33%); grade III: 4 (33%); grade IV: 3 (15%) | 17 (89,5%); grade I: 1 patient (9%); grade II: 3 (15%), grade III: 6 (35%) |

Infectious diseases

| 6 (31,5%); S. Epidermidis 4 (21%); Strept. hominis 1 (5%); CMV 2 (10,5%); C. Albicans 2 (10,5%) | 8 (42%); St. Coagulase negative 7 (36,8%); Shr viridans 1 (5,2%); S. epidermidis 1 (5,2%); Ochronobacter anthrop 1 (5,2%); P. amigognus 1 (5,2%); C. difficile 1 (5,2%); ADN Hepes virus 6: 2 (10,5%)|

In conclusion. We conclude that the use of Cy- Bu compared to the traditional Bu-Cy conditioning may be beneficial for the patients since it allow faster engraftment of the stem cells. These also may help in decreasing the side effect due to the lower levels of cytokines during transplantation period.

HIGH PROPORTIONS OF CD4+CD25+ CELLS IN BLOOD LYMPHOCYTES DETECTED EARLY POST HSCT ASSOCIATE WITH AGVHD AND HERALD ITS SEVERITY

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Institute of Immunology, WROCLAW, Poland

Background. CD4+CD25+ cells have already described as regulatory cells exerting immunosupression. Unexpectedly, we found that these cells are rather elevated at the beginning of aGVHD prior to steroid therapy. Aim. In the present study we investigated whether CD4+CD25+ cells proportions correlated with the severity of aGVHD and influenced chimerism post HSCT in patients receiving non-myeloablative conditioning regimen. Methods. Forty four cases (40 hematological disease and 4 immune deficiencies) transplanted from matched sibling (19) and unrelated donors (25). Three patients with concomitant non-myeloablative and 11 on myeloablative conditioning regimen. The presence of CD4+CD25+ cells in addition to routine lymphocyte profiling was investigated in three time intervals post HSCT (until +30 days, 30-60 days and 60-100 days). Post transplant chimerism was detected with the use of informative genes STR alleles determination between 12’30” days post HSCT. aGVHD were diagnosed clinically and usually the diagnosis was supported by target organ histopathology including immunosupression. Results. Thirty four patients were investigated by one month post HSCT. Mean values SD of CD4+CD25+ cells equaled 9.9±7.7 with a binmodal distribution of individual results allowing dividing the entire group of patients into two subgroups with CD4+CD25+ cells proportion above and below 5%. Twenty and 20 patients had CD4+CD25+ cells below and above 5%, respectively. Twenty eight patients received non-myeloablative conditioning and they were evaluated for the early post-transplant chimerism. Seven out of 16 and 3 out of 12 patients had mixed chimerism in patients groups with high and low proportions of CD4+CD25+ cells. Twenty nine patients receiving myelo- or non-myeloablative conditioning developed aGVHD. aGVHD patients had higher proportions of CD4+CD25+ cells in blood lymphocytes detected at the beginning of aGVHD than those lacking aGVHD and investigated at the similar time post transplant (11,1±5,5 vs 3,7±5,7 respectively, p<0,00007). In addition we found that the level of CD4+CD25+ detected at the beginning of aGVHD correlated with the severity of this complication being full blown at some time post CD4+CD25+ cells measurements. The proportions of CD4+CD25+ cells were 8,4±1,2 and 12,5±2,5 in pts having grade 1 and more severe aGVHD, respectively. The highest proportions of CD4+CD25+ cells in blood lymphocytes were found in patients with GvHD as a limiting factor. Several studies have shown that cells that are involved in initiation and promoting of GvHD are dendritic and T regulatory cells. It has been shown that GvHD is mainly due to of the imbalance (activation or suppression) between these two groups of cells. This disparity somehow relates to the intensity of different chemo-radiotherapy conditioning on the recipients and cytokine storm preceding GvHD events. Our aim was to study the admixture order of busulphan (Bu) and cyclophosphamide (Cy) on the chimerism and engraftment of the dendritic and regulatory T-cells. Methods. Sixty female Balb/c mice were divided in two groups. Group I (Cy-Bu) received Cy (100 mg/kg/day, two days) followed by liposomal Bu (15 mg/kg/day, four days) and group II (Bu-Cy) received the same dose of the drugs but in reverse order. Twenty-two of recipients were transplanted using Sca-1 from Balb/c males. The chimerism and engraftment of dendritic and T-regulatory cells was studied at different time point by EACS and FISH analysis. Weight was followed as an indicator of the mice health status. The spleen weight and cellularity were followed as a sign of cytotoxicity and immune suppression. Results. In both groups, mice weight decreased dramatically on day 0, however the mice gained weight rapidly in group Cy-Bu compared to that seen in group Bu-Cy. The spleen weight and cellularity in group Cy-Bu reached the level of control mice faster (on day +3) compared to that found in group Bu-Cy (on day +6) indicating that the repopulation of lymphoid 1 and B cells in group Bu-Cy was faster than in group Cy-Bu. The expression of CD4+CD25+ cells was slightly lower in patients under AM3 therapy (31,5% vs 42%) (p=0.254). This was due to the lower levels of cytokines during transplantation.

INFLUENCE OF THE ADMINISTRATION ORDER OF BUSULFAN AND CYCLOPHOSPHAMIDE ON THE ENGRAFTMENT AND CHIMERISM IN SYNGENIC STEM CELL TRANSPLANTATION MOUSE MODEL

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Background. Although stem cell transplantation (SCT) is considered to be a curative therapy for malignant and non-malignant diseases, still
grade IV aGvHD and these values were significantly higher as compared to grade I aGvHD cases (15.2%±2.7 vs 8.4%±1.2 for grade IV and I of aGvHD, respectively, p=0.05). Summary. It appears that higher proportions of CD4+CD25+ in blood lymphocytes measured soon after HSCT tend to be associated with mixed chimerism but importantly associated with early manifestation of aGvHD and heralded a severe course of this complication.

0362
SHORTENING OF NEUTROPENIA IN LYMPHOMA PATIENTS AFTER TRANSPLANTATION OF LIN ENRICHED CELLS EXPANDED EX VIVO
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University Hospital Brno, BRNO, Czech Republic

Background. Hematopoietic stem cells are able to regenerate hematopoiensis in all lineages. They are clinically used in transplantation of bone marrow or peripheral blood stem cells (PBSC) after myeloablative regimes of chemotherapy in the patients with diagnosis of leukemia or lymphoma. Aims. The methods of enrichment, isolation, cultivation and expansion of hematopoietic stem cells open the way for specific cellular therapy. In this study, the influence of ex vivo expanded Lin' enriched stem cells on the speed of engraftment was evaluated. Methods. Authors analyzed expansion of hematopoietic stem cells (HSC) selected by immunomagnetic separation of CD34+, CD38− and the culture of serum-free medium in vitro with combination of 5 cytokines (SCF, Flt-3-L, IL-3, IL-6, G-CSF). Cell counts, morphology, immunophenotyping, S-phase, electron microscopy, and biological tests of LTC-IC, CFU-GM and CFU-Meg were analyzed. Clinical protocol was designed based upon the results of in vitro studies. Hematopoietic stem cells were enriched from saphenous products collected from patients undergoing mobilization chemotherapy by Lin' separation and expanded in vitro. Clinical transplantation protocol based on these results was developed. 10 patients with diagnosis of Hodgkin's or non-Hodgkin’s lymphoma indicated for high-dose chemotherapy and autologous PBSC transplantation were enrolled to the protocol. All patients underwent standard PBSC collection, BEAM chemotherapy regimen from day -7 and autologous transplantation at day 0. Besides that, an extra PBSC graft was collected, hematopoietic stem cells were enriched by Lin' procedure and cells were frozen. At day +14, enriched cells were thawed and cultured in the presence of 5 cytokines in serum-free medium. Expanded cells were infused at day 0 to the patients at the escalating dose from 5.10^7 to 3.10^10 cells. Patients were closely monitored, side effects and time to engraftment in leucocytes and platelets was observed. The results were compared to historical controls of 143 patients with diagnosis of lymphoma transplanted with identical BEAM regimen and PBSC grafts. Results. Isolated Lin' cells in culture differentiated, the relative proportion of CD34+ cells decreased below 5% at day +14. Growing number of granulocytic progenitor cells correlates with number of CFU-GM colonies. The highest number of CFU-GM colonies and total cell expansion was observed at day +14 in cytokine combination SCF+IL-3+FLT-3-L and IL-6, which was used in the protocol. The procedure of Lin' cells transplantation was free of side effects in all patients. Engraftment in leucocytes occurred from day +6 to day +9 in the study group. Compared to historical controls, there was a significant shortening of neutropenia to 5.6 days in average and to 5.0 days in patients who received doses over 1.10^9 cells. There was no significant change in the engraftment in platelets (day +10 versus day +11). Conclusions. Hematopoietic stem cells can be enriched from PBSC grafts, cultured and expanded ex vivo, and safely used in the cellular therapy protocols. At higher doses of infused cells, the procedure resulted in shortening of critical period of pancytopenia.

This work was supported by grant IGA NR/0803-3.

0363
CONTINUOUS INFUSION IDARUBICIN AND ORAL BUSULPHAN (IBU) AS CONDITIONING FOR PATIENTS WITH ACUTE MYELOID LEUKEMIA UNDERGOING AUTOLOGOUS STEM CELL TRANSPLANTATION
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Background. One way for reducing the relapse rate after autologous stem cell transplantation (ASCT) in acute myeloid leukemia (AML) in first complete remission (CR) is the adoption of new conditioning regimens. We developed an original conditioning program, named IBu, consisting of the combination of high dose idarubicin (IDA), given at 20 mg/sqm as 3 days continuous infusion from day -13 to -11 and busulfan (Bu) at 4 mg/kg from day -5 to -2, whose feasibility was previously demonstrated in a phase II study on 14 patients (Ferrara et al., THJ 2001). Aims. To report results from a series of 80 AML patients autografted in first CR conditioned with IBu regimen. Patients and Methods. There were 50 males and 30 females with a median age of 53 years (16-77). All patients had non M3-AML autografted in first CR. Karyotype was evaluable in 75 cases, with favourable, intermediate and unfavourable cytogenetics being found in 4, 60 and 11 cases, respectively. All patients received peripheral blood stem cells (PBSC) collected after consolidation plus G-CSF. The median interval between CR achievement and ASCT was 3 months (5-10). The median number of CD34+ cells infused was 6,5x10^6/kg (2,1-29). In patients aged more than 60 years (n=24), IDA and Bu were reduced to two and three days, respectively. Results. One case of transplant related death (1.2%) occurred aged 55 years, due to septic shock. The median number of days with granulocytes <500/cmm and of platelets <20000/cmm was 10 (7-21) and 12 (6-168), respectively. The median number of platelet and blood units transfused was 3 (0-8) and 2 (0-12), respectively. Extra-hematological toxicity mainly consisted of grade WHO III-IV stomatitis (62/88 or 77%) requiring in all cases total parenteral nutrition, while 2 patients had grade III hepatic toxicity and one experienced transient hallucinations. Furthermore, most patients had FU/O, while 3 experienced documented infection. Median days of intravenous antibiotics, required in 75 cases, were 11 (4-28). IVER examination post-ASCT did not reveal any cardiac toxicity. Finally, median time of hospitalization was 28 days (22-49). At the time of writing, 48 patients (54%) are in continuous CR, while 36 have relapsed at a median time from ASCT of 5 months (1-44), with only three patients relapsing after more than one year from ASCT. One patient died in CR from gastric cancer. After a median follow-up for surviving patients of 29 months from ASCT, median overall and disease free survival are 52 months and 48 months, respectively, as shown in the figure. Patients aged more than 60 years did not experience more complications than younger patients. Conclusions. Our data demonstrate the efficacy of the IBu regimen in patients with AML, due to a substantial reduction of relapse rate. The most relevant toxicity of the regimen was severe mucositis requiring TPN.

0364
HIGHER DOSE OF CD4+ T CELLS IN THE ALLOGRAFT AND THE OCCURRENCE OF ACUTE GVHD ARE ASSOCIATED WITH IMPAIRED KIRS EARLY RECONSTITUTION AFTER UNMANIPULATED HLA MISMATCHED/NAPLOIDENTICAL BLOOD AND MARROW TRANSPLANTATION
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Backgrounds. The beneficial effect of killer immunoglobulin-like receptors (KIRs) driven alloreactivity of NK cells had been proved in the T-cell-deplete hematopoietic stem cell transplantation (HSCT), but with the patient’s remission (CR) is the adoption of HSC transplantation (HCT) occurred in a patient aged 50 years due to aGVHD. The results were compared to historical controls of 143 patients with diagnosis of lymphoma transplanted in vitro. The beneficial effect of killer immunoglobulin-like receptors (KIRs) driven alloreactivity of NK cells had been proved in the T-cell-deplete hematopoietic stem cell transplantation (HSCT), but with the patient’s CR. These differences seemed to result from the differences in transplant protocols that utilize different extents of T cells depletion in vitro or in vivo with the existence of antithymocyte globulin (ATG). Aims. The goal of this study was there-
fore to address KIR (i.e. CD158a, CD158b, and CD158e) and CD94, NK2A recovery on the NK cells after HLA-mismatch haploidentical HSCT (with T-cell depletion). Specifically, we wished to assess any differences in KIR recovery that may affect the cytotoxicity and alloreactivity of NK cells, and to compare results with those for HLA-match transplant or HLA-mismatch transplant (with T-cell depletion), as reported by Parham et al. Results. We sequentially evaluated 24 patients before and after HSCT on day +30,+60,+90,+120 and +180, and their donors by flow cytometry. All the patients achieved engraftment and complete donor chimerism after transplantation. All patients were alive and CCR, except 5 who died of transplant-related complications after HSCT; three patients relapsed on days 370, 350, 270 respectively. The recovery of CD4, CD94, NK2A, CD158e, CD158D, and CD158L1 on NK cells in recipients increased first compared with their donor values on day 30 after HSCT (p=0.013, p<0.0001, and p=0.063, respectively), then sequentially decreased from day 60 to day 180, to the donor values. By day 180, NK2A expression on NK cells was still maintained at higher levels compared with their donors’ values. The kinetics of reconstitution of CD158a and CD158b (KIR2DL1) on NK cells was opposite to the kinetics of CD158e recovery, diminishing significantly by day 30 in patients after HSCT compared with their donor values (p=0.016 and p<0.001 respectively), then sequentially increasing by days +60 to +180 after HSCT. However, the kinetics of reconstitution of all KIRs and CD94, CD94: NK2A on day +90 to +180 were similar. From day +90 to +180 the recovery of CD94-NK2A recovery on NK cells after HSCT from day +30 to +180. Meanwhile, the patients were classified into low or high CD4+ cell dose groups based on whether they received less or more than a median CD4+ cell dose of 0.85×10⁹, respectively. There was no significant difference in the incidence of II-IV acute graft-versus-host-disease (GVHD) between the two groups, p=0.0226. NK cells expressed less KIRs in recipients with II-IV GVHD or receiving ‘high’ CD4+ cell dose compared with those with low aGVHD or receiving ‘low’ CD4+ cell dose by day 30/60 after HSCT. Furthermore, the dose of CD4+ cells inversely correlated with the KIRs (CD158a, CD158a+CD158b+, CD158e) on NK cells by day 30 and 60. Summary/Conclusion. These results suggested that both of the T cells in grafts and the occurrence of aGVHD affected the KIRs early reconstitution on NK cells in vivo after HSCT.

0366 MURINE MODEL OF STEM CELL TRANSPLANTATION AND GVHD BASED ON CHEMOTHERAPY CONDITIONING

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Background. Stem Cell Transplantation (SCT) is a curative treatment for a wide range of malignant and non-malignant diseases. In spite of its benefits to the patients, there are numbers of obstacles limiting the wide use of SCT. Because of ethical and technical problems, animal models of SCT are used widely to study basic mechanisms underlying SCT and SCT-related complications such as veno-occlusive disease (VOD) and graft versus host disease (GVHD). The majority of mice models of SCT and GVHD are based on the use of radiotherapy as a conditioning regimen. However, these models can not cover the variety of SCT in clinical settings. Many patients are conditioned with chemotherapy which may affect the occurrence and rate of transplantation related complications. Aims. To establish a murine model of SCT and GVHD using the chemotherapy as a conditioning regimen. Methods. One hundred and twenty female BALB/c mice were divided in two main groups. Group I was conditioned for syngeneic SCT and group II considered for allogeneic SCT. Each group was divided into two subgroups. Cy-Bu subgroups received Cy (100 mg/kg/day for two days) followed by liposomal Bu (15 mg/kg/day four days). Bu-Cy subgroups received the same dose of the drugs but in reversed order. Forty two of the recipients (group I) were transplanted by bone marrow stem cell (Sca-1) of male BALB/c (syngeneic) and forty two recipients from group II were transplanted by bone marrow stem cell (Sca-1) of male C57BL/6 (allogeneic). The chimerism and engraftment were surveyed by FISH analysis. Cytokine levels and immune cell repopulation and dynamics were studied by FACs analysis. Results. Engraftment was established in both groups successfully and started from day +15. Also there were differences in time period of engraftment. In allogeneic group we could show the occurrence of GVHD as well. GVHD has shown symptoms of acute GVHD and occurred between day +30 and day +40 post transplantation. Interestingly this conditioning is not myeloablative and can be considered as non-myeloablative conditioning model of SCT. Summary/Conclusion. We have established a new murine model of SCT using chemotherapy which is compatible and comparable with non-myeloablative model of conditioning in human. This model can also be used to study the basic mechanisms underlying GVHD that might be caused by the effect of the conditioning regimen on different cell sub-populations.
The homing and outgrowth of luciferase gene-transduced hematopoietic cells can be visualized in live animals on sequential time points by bioluminescent imaging (BLI), using a highly sensitive liquid nitrogen cooled charge-coupled camera (CCCD). A safe transduction of bone marrow (BM) hematopoietic cells was optimised for the retroviral vector encoding the Green Fluorescent Protein-luciferase (GFP-Luc) fusion gene. To validate the signal of the BLI in relation to the number of transduced hematopoietic cells, different cell doses were transplanted. There is a linear correlation of the number of cells and the bioluminescence signal in the BM compartment during the first 5 weeks after transplantation. However, after a longer period of time the variation increased between the individual mice. Studying the fate of different transduced murine HSC populations after transplantation into lethally irradiated mice, not-treated BM was compared to Sca-1 positive cells from 5Fluouracil-BM, a technique to enrich for the primitive hematopoietic stem cell. After transplantation of the total cell population with 20% transduced cell, different foci in the BM showed luciferase expression, predominantly in the femurs and sternum. Luciferase activity in mice transplanted with transduced BM cells decreases below detection level after 6 weeks, suggesting that only committed progenitors were transduced in this cell sample. Mice transplanted with transduced Sca-1 positive cells reached a maximum level of luciferase expression at week 4-5 and thereafter a consistent signal during the 7 months, indicative for the activity of the transduced primitive stem cells. The transduction of primitive stem cells was confirmed by a secondary transplantation in which long-term expression of luciferase was observed. These results show that the BLI might be of value to study different populations of hematopoietic stem cells or for monitoring and quantitating the proliferation of locally active hematopoietic cells.
a not suspected germ in 3 patients (Tuberculosis, Citomegalovirus, Aspergillus); 2 erroneous interpretation of the final symptoms etiology in different aspects: we suspect opportunistic infection that was not confirmed in the autopsy, and that was turn to leukemic infiltration (2 cases); compatible clinic with gastrointestinal acute graft-versus-host disease that change to leukemic infiltration (1 case) or fungal infection (1 case). Finally, the autopsy disclosed unexpected involvement of different organs by opportunistic infections in 10 patients. Conclusion. In spite of the advance on the diagnostic procedures, we confirm the profitability of the autopsy as source of valuable information for the clinical manifestations in patients with malignant hematologic illnesses.

0370
CMV INFECTION AND DISEASE AFTER ALLOGENEIC STEM CELL TRANSPLANTATION A SINGLE CENTER EXPERIENCE
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Background/Aims. CMV infection and disease remains a significant cause of morbidity and to some degree mortality after allogeneic stem cell transplantation (ASCT). The aim of this retrospective study was to evaluate the incidence of CMV infection and disease after ASCT to our center and to identify possible risk factors. Patient/Material/Methods. From 1994 to 2004 330 patients underwent ASCT. Conditioning regimens were oral Busulfan 16 mg/kg and IV Cyclophosphamide 60 mg/kg (BuCy2) (N=297) or Cyclophosphamide and ATG (N=10), 28 patients received other conditioning regimens. Graft-versus-host disease (GvHD) prophylaxis was cyclosporine A (CyA) and a conventional short course of Methotrexate. Established acute GvHD ≥ grade II was treated with increased immunosuppression with CyA and corticosteroids, occasionally ATG and other immunosuppressors in steroid refractory GvHD. Blood products were leukocyte depleted or CMV negative to CMV negative recipients. CMV surveillance was routinely performed by measurement of pp65 or PCR 1-2×/week until tapering of immunosuppression. CMV infection was diagnosed by detection of matrix protein pp65 or CMV DNA by PCR in two consecutive samples, which prompted treatment with ganciclovir or foscarine: CMV disease was diagnosed according to the criteria of the 4. International Cytomegalovirus Workshop, Paris, 1993. Results. CMV infection was diagnosed in 22% (N=73) and CMV disease in 6% (N=19). Gastrointestinal (GIT) CMV disease was diagnosed in 11; lung in five, lung and GIT in two and spleen in one case. Two CMV infections were primary, whereas primary CMV disease was not observed. Of the patients who were CMV negative pretx (N=95); infection developed in 3 in patients receiving grafts from CMV positive donors. In the group of patients receiving grafts from family donors (n=220) the incidence of CMV infection and disease was 18.6% and 4% respectively. In the group receiving grafts from matched unrelated donors (MUD) (n=110) these incidences were 29% and 9%. CMV infection and disease were diagnosed at a median of 45 and 46 days post transplant. Late CMV infection, after day 100, was diagnosed in 9 patients. Using logistic regression analysis, the following factors were found to be statistically significant for development of CMV infection and disease: graft from MUD, use of corticosteroids, and CMV seropositive recipient pretx.

Mortality in patients with CMV infection and disease was 47% and 68%, compared to 44% in the total material. Conclusions. In this retrospective single center study the incidence of CMV infection and disease was 22% and 6%. The low incidence of infection might be due to the fact that two positive tests and antiviral treatment were required for diagnosis. Grafts from MUD, use of corticosteroids and seropositive recipient pretx were identified as risk factors. Based on these data, CMV disease did not seem to be the direct cause of death, but the overall mortality is high, reflecting the severity of immunosuppression.

0371
AMPLIFICATION OF AN ASPERGILLUS SPP. SPECIFIC REGION OF THE 18S RNA GENE BY REAL-TIME POLYMERASE CHAIN REACTION ON SERUM SAMPLES AS DIAGNOSTIC TOOL FOR INVASIVE ASPERGILLOSIS IN FEBrILE HAEMATOLOGICAL PATIENTS
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Background. Invasive aspergillosis (IA) is responsible for about 5-10% cases of fever of unknown origin during neutropenia in hematological cancer patients. The diagnosis of invasive aspergillosis is conventionally based on indirect criteria devised by international cooperative study groups (EORTC-MSG) which include the determination of galactomannan (GM) antigenemia. However the precise cut-off values of GM antigenemia are still debated and the sensitivity and specificity are still suboptimal. DNA-based methods have shown potential utility in the diagnosis of invasive fungal infections, and they are sensitive to GM test. Aims. We retrospectively evaluated a new Aspergillus specific real-time polymerase chain reaction (AspRT-PCR) test in the serum of patients affected by hematological malignancies at the onset of fever, in order to evaluate the correlation between the result of AspRT-PCR with the subsequent clinical diagnosis. Methods. Twenty-three patients affected by acute leukemia (n=15), lymphoma (n=7), myelodysplastic syndrome (n=2), chronic lymphocytic leukemia (n=2) were evaluated. They underwent a complete microbiological screening, chest radiograph and CT scan. GM antigenemia >1 was considered positive. AspRT-PCR was performed with an Aspergillus gene-specific Taqman probe and Applied Biosystems 7700 instrument. The Aspergillus-specific probe was designed using Primer Express software, within a conserved region of the 18S rRNA gene of A. fumigatus, A. flavus, A. nidulans, A. niger, A. terreus, A. versicolor, but not homologous to other sequenced pathogenic fungi or mammalian DNA. Thirty sera obtained from healthy volunteers were evaluated as control. Results. All the healthy volunteers were found negative and no cross amplification was observed. According to the EORTC-MSG diagnostic criteria, we classified 5 patients as having probable aspergillosis, 8 as possible aspergillosis and 10 as no aspergillosis. GM antigenemia was positive in 2/5 patients with probable aspergillosis and in 1/10 patients with possible/no infection. AspRT-PCR was positive in all the patients (8/9) with probable infection and in 3 of the 8 patients with possible aspergillosis. Two of the patients classified as no aspergillosis showed a positivity of RT-PCR. Considering the groups of probable infections as true infection and possible/no infection as true negative, the frequency of AspRT-PCR was significantly higher among truly infected patients (67% vs. 13%). The sensitivity was 94% and the specificity was 100%. Specificity was 73% and the positive predictive value 50%. In three of 5 patients with probable aspergillosis, RT-PCR became positive earlier than galactomannan antigen (median 5 days, range 5-9). Conclusions. AspRT-PCR for invasive aspergillosis showed an excellent sensitivity and negative predictive value. Moreover, it could be an earlier marker of fungal infection in comparison with GM, although these results have to be confirmed on larger number of patients. Further studies are needed to disclose factors accounting for false negative results.

0372
INFECTIOUS COMPLICATIONS IN HAEMATOLOGICAL PATIENTS WITH CENTRAL VENOUS CATHETERS: A PROSPECTIVE ANALYSIS OF RISK FACTORS AND ETIOLOGICAL AGENTS
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Background. The use of central venous catheters (CVCs) in haematological patients is associated with various complications, among which infections are the most frequent and life-threatening. Aims. The aim of this single-centre, prospective study was to evaluate the epidemiology and the outcome of catheter-related infectious complications in haematological patients. Methods. Data concerning catheterizations of patients with haematological malignancies were collected between September 2002 and December 2004. The study cohort included 279 patients (137 male and 142 female, mean age 49.7, range 17-75) for a total of 388 catheterizations: 120 acute myeloid leukaemia (43%), 24 acute lymphoblastic leukaemia (6.2%), 99 chronic myeloid leukaemia (26%), 21 with other haematological malignancies (7.5%). In acute leukaemia pts CVCs were used for chemotherapy administration and support therapy during aplasia, while the principal use in lymphoma and multiple myeloma pts was to harvest peripheral blood stem cells. A catheter-related bloodstream infection (CR-BI) was defined by demonstration of the same microorganism both in the catheter and in the peripheral blood cultures, when no other source of infection other than the catheter itself was found. Results. Mean duration of catheterization was 18.8 days, while mean neutropenia with ANC < 0.5×10^9/L during catheter in situ maintained was 7.4 days and mean severe neutropenia with ANC < 0.1×10^9/L was 4.7 days. In particular in acute leukaemia pts mean duration of catheterization was 23.8 days, mean neutropenia with ANC < 0.5×10^9/L was 11.3 days, mean neutropenia with ANC < 0.1×10^9/L was 7.3 days. Exit tunnel infections occurred in 19 cases (2.6 per 1000 catheter days), while catheter-related bloodstream infections
occurred in 49 cases (6.7 per 1000 catheter days). Gram-positive CR-BIs were 93%, among which Staphylococcus epidermidis and Streptococcus were prevalent (58% and 12%, respectively). The remaining were Gram-negative CR-BI, most of which caused by E.coli, Pseudomonas aeruginosa and Enterobacter spp (31%, 28% and 15%, respectively). No fungal CR-BI was diagnosed. During hospitalization two patients (0.7%) died due to their haematological disease; catheter removal because of repeated nosocomial complications was necessary in 14 cases (3.61%), of which 6 showed CR-BI. At univariate analysis, significant risk factors for CR-BI were number of days/catheter (p < 0.0001), chemotherapy dose (high vs. standard dose; p < 0.015), duration of neutropenia (p < 0.001) and thrombocytopenia (p < 0.001). At multivariate analysis, only days/catheter and duration of neutropenia appeared significant risk factors for CR-BI. Conclusions. The incidence of CR-BI is greatly increased by risk factors connected to haematological diseases and consequent chemotherapy administration, such as duration of catheterization, neutropenia and thrombocytopenia. In particular, patients affected by acute leukaemia are at a higher risk for CVC-related infections due to the use of aggressive and/or high-dose chemotherapy cycles during induction and salvage therapies, and a longer duration of severe neutropenia.

0373 RESPIRATORY VIRAL INFECTIONS IN IMMUNOCOMPROMISED PATIENTS

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Background. Acute respiratory viral infections are generally self-limiting, but can lead to morbidity and mortality in immunocompromised patients particularly in the setting of bone marrow transplantation. Mortality in previous series has varied from 30 -100%. Aims. 1. To establish the incidence of respiratory viral infection in our unit. 2. To identify high risk patients requiring treatment and determine outcome. Methods. During the study period, July 2003-June 2005, all symptomatic patients had samples of respiratory secretions, nasopharyngeal aspirate (NPA) or broncho-alveolar lavage evaluated for respiratory viruses. Symptoms included cough, fever and coryza. Results were phoned to the referring doctor. Results. There were 40 positive results in thirty-eight patients. The following viruses were identified and patients were commenced on appropriate therapy; oseltamivir for influenza A or B; Nebulised ribavirin for 5 five days and alternate day intravenous immune globulin for RSV and parainfluenza.

<table>
<thead>
<tr>
<th>Virus</th>
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<tr>
<td>RSV</td>
<td>11 (29%)</td>
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<tr>
<td>Parainfluenza 2</td>
<td>2 (5%)</td>
</tr>
<tr>
<td>Parainfluenza 3</td>
<td>16 (42%)</td>
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<tr>
<td>Influenza</td>
<td>8 (21%)</td>
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<tr>
<td>Influenza B</td>
<td>1 (3%)</td>
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<td>Adenovirus</td>
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There were no deaths associated with respiratory virus infection during the study period (July 2003-June 2005) One patient with chronic GVHD required non invasive ventilation for acute respiratory distress due to RSV. Two patients required a second course of treatment due to persistent symptoms. Two transplants were deferred due to Influenza A. Conclusion. A high index of suspicion with early investigation and prompt isolation and treatment reduces the morbidity and mortality associated with these infections in immunocompromised patients. The presence of lymphopenia, GVHD, and steroid administration are risk factors for poor outcome.

0374 REVERSE SEROCONVERSION OF HEPATITIS B VIRUS AFTER ALLOGENIC OR AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background. Reactivation of hepatitis B virus (HBV) in patients with anti-hepatitis B surface antigen antibody (HBsAb) has been known as reverse seroconversion, and recognized as a rare complication after hematopoietic stem cell transplantation (HSCT). However, its precise incidence has yet to be fully elucidated. Aims. We retrospectively analyzed the incidence of HBV reverse seroconversion in patients undergoing allogeneic or autologous HSCT for hematologic diseases. Patients and Methods. Eighty-three patients undergoing allogeneic HSCT (allo-HSCT: n=47) or autologous HSCT (auto-HSCT: n=36) between March 1992 and December 2005 were HBsAb-positive before transplant, and could be evaluated. Median age was 44 years-old (range 18-61) in allo-HSCT recipients, and 52 years-old (range 26-66) in auto-HSCT recipients. Diagnoses were acute leukemia in 25, non-Hodgkin’s lymphoma (NHL) in 22, multiple myeloma in 20, chronic myelogenous leukemia (CML) in 11, and aplastic anemia in 1. Stem cell sources were bone marrow or peripheral blood stem cells (PBSCs) from related donor (n=23), bone marrow (n=30) or cord blood (n=2) from unrelated donor for allo-HSCT, and PBSCs (n=28) for auto-HSCT. Only 2 of 55 allogeneic donors were HBsAb positive. For conditioning, patients received myeloablative (n=4) or reduced-intensity regimen (n=11) for allo-HSCT, and high-dose melphalan regimen (n=17), MCVAC regimen (n=9), or total body irradiation-based regimen (n=2) for auto-HSCT. Results. Three of 55 patients (5.5%) and 3 of 28 patients (10.7%) experienced HBV reverse seroconversion after allo- and auto-HSCT, respectively. Time to reverse seroconversion from HSCT was 7.8, 10.5, and 53.6 months in 3 allo-HSCT recipients, and 4.6, 6.1, and 6.6 months in 3 auto-HSCT recipients. Underlying diseases were acute leukemia, CML, multiple myeloma in allo-HSCT recipients, while multiple myeloma in 3 auto-HSCT recipients. In 4 (allo-HSCT 3, auto-HSCT 1) out of 6 patients, clinical hepatitis was diagnosed. Conclusions. HBV reverse seroconversion after HSCT is not infrequent, and close HBV monitoring is strongly recommended in HBsAb-positive patients. Furthermore, it is suggested that allo-HSCT recipients might be at higher risk of developing clinical hepatitis due to HBV reverse seroconversion than auto-HSCT recipients.

0375 HEPATITIS AND REACTIVATION OF HBV DURING TREATMENT OF DLBCL PATIENTS: AN UNDERSERAINED EVENT

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Background. Hepatitis due to HBV reactivation after high dose chemotherapy for cancer patients is a well-recognized complication in chronic HBV carriers. The clinical consequences of hepatic injury range from asymptomatic liver dysfunction to massive hepatic necrosis and death by liver failure. Aims. The aim of this study is to compare the occurrence of hepatitis and HBV reactivation in Diffuse Large B Cell Lymphoma (DLBCL) patients with distinct HBV serologic pattern. Methods. We reviewed the medical files of sixty two patients with DLBCL followed since 1997 until now. All patients were treated according to the IFM study of the lymphoma group. We defined 3 groups of patients based on the serologic HBV pattern at diagnosis. The first one included patients who had no antibodies anti-HBc and no antigen HBs (AbHBc- / AgHBs-), the second group included patients who had antibodies anti-HBc but no antigen HBs (AbHBc+/AgHBs-), the third group included patients who carried antigen HBs (AgHBs+). The endpoint of this study was the occurrence of hepatitis and HBV reactivation in Diffuse Large B Cell Lymphoma (DLBCL) patients with distinct HBV serologic pattern. Results. We reviewed the medical files of sixty two patients with DLBCL followed since 1997 until now. All patients were treated according to the IFM study of the lymphoma group. We defined 3 groups of patients based on the serologic HBV pattern at diagnosis. The first one included patients who had no antibodies anti-HBc and no antigen HBs (AbHBc- / AgHBs-), the second group included patients who had antibodies anti-HBc but no antigen HBs (AbHBc+/AgHBs-), the third group included patients who carried antigen HBs (AgHBs+). The endpoint of this study was the occurrence of hepatitis and HBV reactivation in Diffuse Large B Cell Lymphoma (DLBCL) patients with distinct HBV serologic pattern. Results. We identified forty one patients in the AbHBc-/AgHBs- group, thirteen in the AbHBc+/AgHBs- group and four in the AgHBs+. None of the patients had hepatitis before treatment. Seventeen patients developed hepatitis during or after the treatment: Nine in AbHBc-/AgHBs- group (9/41 = 22%), four in AbHBc+/AgHBs- (4/13= 31%) and four in AgHBs+ group (4/4 = 100%). The rate of hepatitis was significantly higher in AgHBs+ group than in AbHBc-/AgHBs- group (100% vs 21%, p<0.001) and than in AbHBc-/AgHBs- group (100% vs 31%, p=0.015). A trend to higher rate of hepatitis development was observed in AbHBc+/AgHBs- group comparatively to AbHBc-/Ag HBs- (81% vs 21%, p=0.076). All the patients in the AgHBs+ group developed HBV reactivation and severe hepatic complications. Three of them died from liver failure. Interestingly, one patients of the AbHBc+/AgHBs- developed a HBV reactivation with acute hepatitis (ALT >1000 IU). In the others patients hepatitis was transient, ALT levels did not exceed 300 UI and no seroconversion occurred. Conclusions. Despite the small number of patients, we observed a significant higher risk to develop severe hepatitis in DLBCL patients with AgHBs+ (chronic HBV carriers). In those cases, hepatitis was due to HBV reactivation and associated with a high mortality. Hepatitis related to HBV reactivation occurred also in AbHBc+/AgHBs- patients, who have presumed resolved hepatitis B. These results emphasize the need for a careful follow-up for chronic HBV carriers and patients with pre-
SUMMED RESOLVED HEPATITIS B. PROPHYLAXIS FOR HBV REACTIVATION SHOULD BE ADMINISTERED TO ALL CHRONIC HBV CARRIERS BEFORE CHEMOTHERAPY REGARDS TO THE HIGH RISK OF SEVERE HEPATITIS AND HBV RELATED DEATH. THE OPPORTUNITY OF VIRAL PROPHYLAXIS IN PATIENTS WITH ANTIHBc ANTIBODIES BUT NO HBs ANTIGEN DESERVES TO BE FURTHER INVESTIGATED.

0376

ORAL VALGANCYCLOVIR IS AN EFFECTIVE PRIMARY PREEMPTIVE THERAPY OF CYTOMEGALOVIRUS DISEASE IN PATIENTS OF ALLOGENEIC STEM CELL TRANSPLANT

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Cytomegalovirus (CMV) infection is a common complication after allogeneic SCT. Valgancyclovir hydrochloride (VALCYTE-VGC) is a prodrug of ganciclovir, orally available, that has been used in CMV infection in high-risk solid organ transplants (donor positive, recipient negative); there were only a few data about this drug in allogeneic SCT. The primary aims of our study were the assessment of efficacy and safety of VGC as preemptive therapy of CMV disease after allogeneic stem cell transplantation. This study is ongoing and here we are reporting the preliminary results. During a five-month-period VGC was administered to 10 consecutive patients (pts) with a CMV infection which was diagnosed in a median time of 86 days (range 59-480) from transplant. There were 6 males and 4 females (myelofibrosis 2, leukemia 4, myeloma 2, lymphoma 2). The median age was 55 years (range 43-66); 7/10 pts underwent stem cell transplantation from unrelated and 3/10 from related donors; 7/10 pts received a reduced intensity conditioning regimen (RIC); CMV prophylaxis consisted in acyclovir in all cases. Pre-transplant CMV serology showed that in 100% of cases either recipient and/or donor were positive (D+/R+ = 5/10, D-/R+= 5/10). At the onset of CMV infection 9/10 (90%) pts have an acute or chronic graft versus host disease for which were received therapy including prednisone plus other drugs. The pp65 antigenemia assay were positive in all cases with a median number of positive nucleic of 21±35. The starting treatment dosage of VGC was 900 mg twice a day and it was continued until the CMV antigenemia and PCR became negative in two consecutive samples. All 10 cases obtained a clearance of antigenemia after a median of 8 days of VGC therapy (range 5-16 days); viremia became negative in all cases. The median length of maintenance therapy with VGC (900 mg once-daily) was 21 days (range 8-32). Only one patient developed a mild deterioration of renal function that required dose adjustment (VGC 450 mg once-daily). None of the pts developed gastrointestinal disorders; mild anemia was reported in 3/10 (30%) pts, neutropenia in 5/10 (50%) pts and thrombocytopenia in 4/10 (40%). Conclusions: 1) Preventive therapy with VGC after related and unrelated allogeneic SCT seems to be safe and effective (with a rapid clearance of antigenemia and viremia). 2) The simple once or bi-daily VGC regimen can improve the compliance of the pts. 3) Regular blood counts should be performed to early detect cytopenia. 4) The optimal dose and duration of VGC therapy in this setting need to be established with additional prospective studies.

0377

BAL AS DIAGNOSTIC TOOL IN SEVERE PNEUMONIA IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES: SINGLE CENTER REPORT ON 16 PATIENTS

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Pneumonia is one of the most frequent life-threatening complications in patients affected by hematological malignancies despite recent improvements in support therapy; in these patients a timely identification of the microbiological agent is crucial. The aim of this study was to evaluate the utility of broncho-alveolar lavage (BAL) in etiological diagnosis of pulmonary infections in this setting. Over 2 years period 16 patients affected by hematological malignancies (2 Myeloma, 1 Essential Thrombocytemia, 5 Non Hodgkin Lymphoma, 4 Acute Myeloid Leukemia, 1 Myelofibrosis, 2 Chronic Lymphocitic Leukemia, 1CML BC) age 58-76 years, showing clinical and radiological signs of Severe Pneumonia and failing to respond to antimicrobial therapy were studied. Together with initial routine serological and microbiological diagnostic tests; blood on urine and sputum (if available) broncho-alveolar lavage (BAL) was performed to identify the etiological agent. According to ATS (American Thoracic Society) criteria a bacterial cut-off > 10^4 CFU/mL or the isolation of a pathogen which doesn’t ordinarily colonize the upper respiratory tract (M. tuberculosis, Pneumocystis J. Legionella sp, Aspergillus sp) defined infectious pneumonia. Results: the final diagnosis obtained by means of BAL among the 16 patients enrolled was: a) infectious pneumonia in 7 patients: the etiological agent was 2 polimicrobial infections (Mycobacterium T plus E.Coli, Mycobacterium T plus Pseudomonas), 2 Aspergillus sp (1 diagnosed by galactomannan detection on BAL and serum), 1 MRSA, 1 Corynebacterium sp. b) non infectious lung disease in 6 patients with alternative diagnosis: 2 alveolar drug damage, 2 BOOP, 1 T cell lymphoma, 1 bronchial infiltration of CIL c) 3 unknown diagnosis. Routine laboratoristic results were diagnostic only in one case (serum galactomannan detection). In conclusion: discrimination between infectious and non infectious diseases that mimic pneumonias is laborious namely in hematological patients; in our experience BAL procedure had a substantial impact on the etiological diagnosis and allowed a change of therapeutic strategy in 10 of 16 cases (62%).

0378

SURVIVAL AND DISEASE COMPLICATIONS OF THALASSEMIA MAJOR - 14 YEARS EXPERIENCE AT KING ABDULAZIZ UNIVERSITY HOSPITAL, JEDDAH, KSA

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Background. Treatment of thalassemia major is complex, expensive and requires a multidisciplinary approach. Optimal clinical care is demanding and expensive but achievable. In spite of medical treatment improving dramatically, complications and deaths still occur. Aim. To assess the prevalence of disease and survival and disease complications among patients with thalassemia major at our center. Methods. A retrospective chart review was done of all patients diagnosed as Thalassemia Major (TM) between 1990 and 2004. The patients were followed and treated at King Abdulaziz University Hospital (KAUH), an academic tertiary care medical center. All 360 patients (208 males & 157 females) were transfusion dependent since early childhood and treated with parenteral Deferoxamine. Approximately 98% were B-TM and 2% were HbE_o. The data had been collected by means of specially prepared forms (from Hematology Clinic, Day Care and Medical Records Department). The mean of serum ferritin has been available for all patients yearly. Comparison of ferritin levels between groups was performed by Student’s t test. Results. Out of 360 patients, 295 (81.4%) patients were alive, 27 (7.2%) patients had died, 15 (4.2%) patients underwent BMT and 25 (6.9%) patients’ follow-up were lost. Twelve (3.3%) patients died from heart disease. 7 (1.9%) patients died from infections, all patients were splenectomised. The serum ferritin levels for patients who died were significantly higher than for those patients who survived (7,500 vs. 3,200, p<0.001). Conclusions. Cardiac constitutes the first important cause of death followed by infection. Infection among thalassemics is still a risk factor which needs to be addressed carefully. Splenectomized thalassemic patients required special attention to avoid and prevent infections. Infection among thalassemics is still a risk factor which needs to be addressed carefully. Splenectomized thalassemic patients required special attention to avoid and prevent infections.

Table 1.

<table>
<thead>
<tr>
<th>Causes of Death</th>
<th>Number</th>
<th>Percentage</th>
<th>Mean Age</th>
<th>Age Range</th>
<th>Mean S. Ferritin (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac</td>
<td>12</td>
<td>3.3</td>
<td>20</td>
<td>16-24</td>
<td>7500</td>
</tr>
<tr>
<td>Infection</td>
<td>7*</td>
<td>1.9</td>
<td>14</td>
<td>10-18</td>
<td>2400</td>
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<tr>
<td>Endocrine</td>
<td>3</td>
<td>0.8</td>
<td>24</td>
<td>21-25</td>
<td>6000</td>
</tr>
<tr>
<td>Liver Disease</td>
<td>2</td>
<td>0.5</td>
<td>21</td>
<td>20-22</td>
<td>4000</td>
</tr>
<tr>
<td>Thrombosis/bleeding</td>
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<td>0.2</td>
<td>17</td>
<td>12</td>
<td>3500</td>
</tr>
<tr>
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<td>2</td>
<td>0.5</td>
<td>19</td>
<td>18-20</td>
<td>3000</td>
</tr>
<tr>
<td>Total</td>
<td>27</td>
<td>7.2</td>
<td></td>
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</tbody>
</table>

*Specifcithated patients.
INCIDENCE AND RISK FACTORS OF INVASIVE FUNGAL INFECTIONS IN 246 PATIENTS UNDERGOING RELATED OR UNRELATED ALLOGENIC BONE MARROW TRANSPLANTATION.

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University Hospital, UDINE, Italy

Introduction. Allogeneic bone marrow transplantation (BMT) is increasingly used to treat hematologic diseases. Invasive fungal infections (IFI) remain an important cause of morbidity and mortality in this setting. Patients and Results. To evaluate the epidemiology, outcome and risk factors of proven or probable IFI in allogeneic BMT recipients, we retrospectively examined the medical records of 246 consecutive adult patients (pts) who underwent allogeneic BMT (150 pts from related and 96 from unrelated donor) at our Department between 1992 and 2004; 193/246 (78%) pts received a myeloablative conditioning regimen and 53/246 (22%) a non myeloablative one. The median age of patients was 42 years (range 19-66). We identified 31 cases of IFI with an overall incidence of 13%; the incidence after related BMT (R-BMT) was 8% (12/150) while it was 20% (19/96) after unrelated BMT (UR-BMT) (p<0.05). The incidence was the same in the myeloablative (24/193, 12%) and non myeloablative (7/53, 13%) setting. IFI occurred after a median of 41 days from BMT (range 5-1440). There were 28 cases with proven or probable IFI (Aspergillus 22, Candida 4, Fusarium 1, Mucor 1) and 3 cases with possible IFI (all with lung localization). The sites of infection were: lung only 21/31 (69%), CNS 4/31 (13%), multiple sites 6/31 (19%); 13/31 (42%) cases occurred during pre-engraftment phase while 18/31 (58%) occurred after engraftment (with 12/18 cases after day 100). Advanced hematologic disease (relapsed or refractory) at time of transplant, history of pre-transplant IFI, presence of acute or chronic graft-versus-host disease (GVHD), and neutropenic fever were independent risk factors (p<0.05). In the UR-BMT setting the incidence of IFI was significantly higher in patients who received a combination of immunosuppressive agents in the conditioning regimen (ATG ≥ Fludarabine ≥ Campath). Overall Survival after 100 days from diagnosis of IFI was only 20% and 22/31 (70%) deaths directly IFI related were reported. Conclusions. 1) IFI is an important problem in R and UR-BMT and it is significantly more frequent of nonrelapse mortality. 2)Aspergillus sp. remain the most important aetiologic agent. 3)The incidence of IFI in UR-BMT is significantly higher than in R-BMT probably as a result of intensive immunosuppressive conditioning regimens in this setting. 4)IFI can develop late after engraftment (after day 100 from transplant) and without neutropenia. 5)Presence of GVHD, status of hematologic disease (relapsed/refractory) at transplant and history of pre-transplant IFI are important predisposing factors. 6)Retrospective studies, like this one, can be useful in order to identify high-risk BMT patients for which targeted and more effective strategies should be explored to prevent and treat IFI.
Invasive aspergillosis is a serious complication with high mortality in allogeneic stem cell recipients and patients with acute leukaemia. Construction work close to a haematology ward is a known risk factor for aspergillus infections. At the Helsinki University Central Hospital, heavy construction work was performed from mid October until the end of year 2005 immediately adjacent to the 13-bed HEPA-filtered stem cell transplantation ward, located on the ground floor of the building. A protective barrier was built around three close-by ventilation ducts and around the construction area. The function of the air filters was followed by daily checking of the air pressure of ventilation channels. No increase in the pressure was seen. Regular surveillance sampling was performed in the ward. Particle counts were measured for particles above 0.3 microns in all patient rooms five times a week using Particle Scan Pro (IQ Air). The median particle count was 65–420 particles/litre. One peak of 1084 particles/litre was noticed. This was associated with heavy drilling during reconstruction work inside the hospital, four floors above the ward. The particle counts of the outside air at the hospital main entrance were significantly higher, between 110806 and 185645 spores/litre. Sampling for fungal spores was performed with a Surface Air Sampler, SAS 100 (ipi International, Italy). The samples were taken once a week from five different locations; three patient rooms, the construction area and the hospital main entrance. The samples from patient rooms were negative on 31 and positive on two occasions, one with Aspergillus niger (1 CFU/m³) and the other with nonpathogenic, environmental fungi. The samples from the construction area and the hospital main entrance were all positive, with 2-21 (median 9) CFU/m³ and 1-61 (median 7) CFU/m³, respectively. To rule out colonisation of the patient rooms and the patients, fungal cultures were performed. Surface samples from three different patient rooms were obtained once a week using contact plates. Of the 33 samples, 23 were negative and seven were positive but only for nonpathogenic fungi. Three samples were positive for aspergilli, two with Aspergillus fumigatus and one with Aspergillus niger. Swab samples were taken from both nares and the mouth of all patients and cultured for fungi on three occasions. All 70 nasal samples from 24 patients were negative. Of the 35 mouth samples, 18 were negative. Of the positive samples, 16 grew yeasts and one grew Aspergillus niger. This patient had been diagnosed with pulmonary aspergillosis prior to the beginning of the construction work. 55 patients were treated on the ward during this period. 15 allogeneic and 7 autologous stem cell transplantations were performed a prospective randomized phase II comparison study to assess the management of haematological and oncological patients, but is not exempt from complications. Aims. We describe our experience with Hickman catheters in a tertiary care center in Spain from 1992 and 2005, trying to quantify the complications and characterize them. Patients and Methods. A retrospective analysis was performed on 248 consecutive double lumen Hickman-Broviac catheters (109 of fine diameter 9F- and 139 of gross diameter 13F)-inserted in 190 patients with haemopathies (151) or solid tumours (39). Catheters were inserted by the patient’s haematologist under fluorescopic guidance. Results. Six early complications occurred, being the most relevant a subcutaneous tunnel necrosis. In 39.3% of the catheters a late complication was observed: 28.1% infectious, 9% mechanical, and 2% both of them. The infection location: 76.2% bloodstream, 13.8% exit site and 10% tunnel infection. The most common microorganisms isolated were coagulate-negative Staphylococcus (38.8%), Pseudomonas sp. (15.8%), Klebsiella sp. (10%), Escherichia coli (8.8%) and other Gram negatives (20%). The main mechanical complications were: accidental removal (4%), device breaking and symptomatic thrombosis (1.6% in each case). The total catheter days at risk (CVC-days) were 20,902 (median: 54 days, range: 2-486 days). The overall complication rate was 4.9/1000 CVC-days (infectious rate 3.6/1000 CVC days: mechanical rate: 1.3/1000 CVC-days). The complication rate of gross catheters was 5.9/1000 CVC-days and 5.3/1000 CVC-days in the fine diameter devices. Complications were less likely to develop in catheters inserted in patients with haemopathies compared with those with solid tumours (7.2/1000 vs 7.4/1000 CVC-days, p = 0.002). Conclusions. In our experience, the Hickman catheter-related complication rate was 4.9/1000 CVC-days. Complications are more frequent in patients with solid tumours, but we did not found an statistical significance in the risk of complication related to the catheter diameter.
Hodgkin’s lymphoma (NHL, 12 patients) were stratified by disease and randomly allocated to receive (prophylaxis group, 21 patients) or not receive (control group, 19 patients) prophylactic antimicrobials just prior to or administration of high-dose chemotherapy. Prophylactic antimicrobials consisted of ciprofloxacin (500 mg twice daily p.o.), fluconazole (100 mg twice daily p.o.) and acyclovir (400 mg every 8 h p.o.), starting 1 day before high-dose chemotherapy (high-dose melphalan for MM and BEAM for NHL) and continuing until absolute neutrophil count reached 500/mm³ after nadir or infection occurred. Lenograstim 5 μg/kg/day was given from day 1 of ASCT. Results. At least one episode of fever occurred in 15/19 (79%) patients in the control group, compared with 12/21 (57%) patients in the prophylaxis group (p=NS). Microbiologically or clinically documented infections occurred in 4 patients (21%) in the control group, but none in the prophylaxis group (p=NS). Documented infections in the control group included 3 staphylococcal bacteremias and 1 herpes skin infection. No deaths, invasive fungal infections, or serious adverse events occurred in either group. The median duration of fever (9 days in the control group and 11 days in the prophylaxis group), days of neutrophil engraftment (9 days in the control group and 11 days in the prophylaxis group), and hospital stay after ASCT (19 days in both groups) did not differ between the groups. Median time to neutrophil engraftment was 10 days in both groups and median time to platelet engraftment was 11 days in the control group and 12 days in the prophylaxis group. Summary/Conclusion. This small-sized prospective randomized phase II comparison showed no beneficial effect of antimicrobial prophylaxis in ASCT.
prophylaxis only appears delay events as suggest the largest period of latency observed in allograft patients.

0389
AUDIT OF THE USE OF CT SCANNING AND RISK STRATIFICATION IN THE DIAGNOSIS OF INVASIVE FUNGAL INFECTIONS IN PATIENTS WITH HAEMATOLOGICAL MALIGNANCIES
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1West Suffolk Hospital, BURY ST. EDMUNDS, United Kingdom; 2Dept.Haematology/Addenbrooke’s Hospital, CAMBRIDGE, United Kingdom; 3Dept.Radiology, Addenbrooke’s Hospital, CAMBRIDGE, United Kingdom

Background. Invasive fungal infections (IFIs) are a major cause of mortality and morbidity in neutropenic patients, but accurate early diagnosis remains difficult. Bronchoscopy is often problematic in such patients. As an alternative, computerised tomography (CT) examinations of the chest are non-invasive, readily available and show characteristic appearances if performed early in the course of the disease. The British Society for Medical Mycology (BSMM) has proposed standards for the diagnosis of patients with IFIs. Aims. We aimed to audit the radiological diagnosis of IFIs following these guidelines. In addition, we sought to assess the impact of risk stratification on the diagnosis of IFIs. Risk stratification models divide patients into low and high-risk groups. High-risk categories for invasive pulmonary aspergillosis are: - Neutropenia (neutrophils <= 0.2×10⁹/L) due to intensive chemotherapy, if prolonged for > 21 days or concomitant steroid administration. - Post-allogeneic transplant if engraftment delayed or on steroids for graft versus host disease (GvHD). - Fungal spore exposure in neutropenia or recent invasive mould infection. Methods. 32 febrile, neutropenic patients initially treated with triple antibiotic therapy were enrolled in the study. CT chest studies were requested for the following indications: - Fever unresponsive to 72 hours of antibiotics (18/39), or to 7 days of antibiotics in presence of another probable bacterial focus of infection (3/39) - Respiratory symptoms or signs (17/39, 8 with chest radiograph changes). - Positive fungal sputum cultures (1/39) CT was performed a median of 16 hours (range 1 hour - 45 hours) following request. A total of 39 examinations were performed in 32 patients. Patient details are outlined in Table 1. Statistical significance for a difference in the incidence of fungal infections between the low and high-risk groups was tested using the chi-square test. Results. CT diagnosis of pulmonary IFI was made in 11 patients (2 of whom had IFI with bacterial super-infection). Other diagnoses made were bacterial bronchopneumonia (9/39), atypical chest infection (1/39), GvHD (3/39), viral pneumonitis (2/39) and Pneumocystis carinii pneumonia (1/39). Bronchoscopy was performed in 4 cases, one of these was positive for Candida albicans. One patient died of IFI during the audit period. Patient risk was classified as high (HR: 27/39) or low (LR: 12/39) for invasive mould infections according to the Martino and Viscoli criteria. IFI was not confirmed in a single LR case, but was diagnosed in 11/27 HR patients (p=0.009). If CT imaging had been limited to high risk patients who met above criteria, a total of 12/39 examinations (31%) could have been avoided without missing a single case of IFI. Conclusion. Identification of patients at low risk of IFI by risk stratification may save resources, and reduce the use of empirical antifungal agents. CT is a useful diagnostic tool in IFIs.

Table 1. Patient characteristics (n=32)

<table>
<thead>
<tr>
<th>Category</th>
<th>Count</th>
</tr>
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<tbody>
<tr>
<td>Allograft recipients</td>
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</tr>
<tr>
<td>Autograft recipients</td>
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</tr>
<tr>
<td>Chemotherapy for NHL or CLL</td>
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</tr>
<tr>
<td>Chemotherapy for ALL</td>
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</tr>
<tr>
<td>Chemotherapy for CML blast crisis</td>
<td>1</td>
</tr>
<tr>
<td>Chemotherapy for AML</td>
<td>11</td>
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</table>

Conclusions.

0390
CLINIC-ANALYTIC PROFILE AND USEFULNESS OF BONE MARROW SMEAR EXAMINATION AND SEROLOGICAL METHODS IN PATIENTS WITH VISCERAL LEISHMANIASIS EXPERIENCE OF OUR CENTER
E.M. Donato,1 J. Marco,2 R. García-Boyero,1 M. Guinot,3 C. Mas-Ochoa,1 A. Escolá,1 I. García, E. Herrera-De Pablo, M. Más, T. Gozalbo,1 J. Amelia, G. Cañigral
1General Hospital of Castellón, CASTELLON, Spain

Background. The Mediterranean area is an endemic region of visceral leishmaniasis (VL). With the advent of human immunodeficiency virus (HIV) infection, the number of cases of VL has dramatically increased in this area over the last years, mainly in adults. Aims. To analyze the clinic- analytic profile and usefulness of methods used in diagnosis of visceral leishmaniasis in our center. Patients and Methods. A total of 58 cases of VL were overviewed retrospectively from January 1989 to September 2005. Sex, age, clinic and analytic profile, diagnostic methods and HIV infection were studied. The median age was 29 years ([range: 8-81] years) with 72% males (n=42) and 28% females (n=16). At diagnosis 97% presented fever (n=56), 91% splenomegaly (n=53), 71% hepatomegaly (n=53) and 19% pancytopenia. In 76% of patients (n=44) the hemoglobin was <100 gr/L, 34% (n=20) neutrophil count <1000 cells/mm³ and 41% had thrombocytopenia (<100x10⁹/L). HIV infection affected 21 patients (56%) and the median hemoglobin of our series was 89 gr/L (range 53-135). The methods used in our center for the diagnosis of VL are bone marrow smear examination and serology methods (Indirect immunofluorescent antibody test (IFAT) and ELISA). The statistical analysis was performed using the program SPSS v10.0. Results. The serodiagnosis of VL was positive in 26 cases and direct examination of the bone marrow smear yielded the diagnosis in 52 cases. The sensitivity of serologic studies was significantly lower in HIV(+) than in HIV patients (p=0.031). The 6 cases with negative examination of the bone marrow smear were HIV + (p=0.057). Conclusions. The diagnosis of VL should be based in a direct examination of the bone marrow smear in combination with another diagnostic procedure. A negative serology is possible in HIV+ patients. When PCR is not available the diagnosis method of choice in HIV/leishmaniasis co-infected patients is direct examination of the bone marrow. In HIV(-) patients the sensitivity of serological methods is better than that of direct examination.

<table>
<thead>
<tr>
<th>Category</th>
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<tr>
<td>Female</td>
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<td>Male</td>
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<td>Female</td>
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<td>Negative</td>
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<tr>
<td>Positive direct examination of bone marrow smear</td>
<td>52%</td>
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<tr>
<td>Negative serodiagnosis of VL</td>
<td>26%</td>
</tr>
</tbody>
</table>

Conclusions.

11th Congress of the European Hematology Association
Thrombosis

0391
DEEP VEIN THROMBOSIS AND PULMONARY EMBOLISM CAN BE TREATED AT HOME IN PATIENTS WITH CANCER
University of Palermo, PALERMO, Italy

Background. Outpatient treatment of deep vein thrombosis (DVT) has become a common practice in uncomplicated patients. Scanty data are presents in patients with comorbidity (such as cancer) or concomitant symptomatic pulmonary embolism (PE). Cancer patients with Venous Thromboembolism (VTE) are often excluded from home treatment because of high risk of bleeding and recurrent thrombosis. We tested the feasibility and safety of the home-treatment program in cancer patients with acute VTE. Material and Methods. Consecutive cancer patients having a confirmed episode of DVT or PE were treated as outpatients unless they required admission for other medical problems, were actively bleeding or had pain treated with i.v. narcotics. As anticoagulants, patients received standard therapy with Low Molecular Weight Heparin (LMWH) followed by warfarin or LMWH alone, at therapeutic dosages; all of them were treated for 6 months. At the index visit, an educational program for self-injection and clinical surveillance was implemented. Results. Over a period of 3 years, 207 patients with cancer and acute VTE (139 with DVT and 68 with PE) were evaluated; 56 (17.4%) of them had metastatic disease. Treatment with standard anticoagulation (LMWH followed by warfarin) was given to 106 (51.2%) while LMWH alone to 102 (48.8%) patients. One hundred and twenty-seven patients (61.3%) (91 with DVT and 36 with PE) were entirely treated at home. In the remaining patients, reasons for hospital admission (n. 80) were poor compliance (22, 27.5%), concomitant serious illness (52, 65%) and refusal of home-treatment (6, 7.5%). There were no differences between patients treated at home and those hospitalized with regard to gender, mean age, site of cancer, presence of metastases and choice of anticoagulants (Table).

After 6 months, recurrent DVT, PE and major bleeding occurred in 6.5%, 6.5% and 1% of patients treated at home, and 5.3%, 9.3% and 2% of those hospitalised. These differences were not statistically significant (p=0.58). Twenty-seven patients (33%) in the hospitalized group and 33 (26%) in the home-treatment group died as a consequence of thrombosis or bleeding. Aims. The aim of the present study was to assess the LMWHs global antithrombotic activity by using a rather physiological-relevant system. For this purpose we used the Thrombogram-Thrombinscope assay, a dynamic assay which describes all the phases of thrombin generation (TG) process (initiation, amplification and inhibition of TG as well as the integral amount of generated thrombin). Methods. TG was assessed after tissue factor (TF) pathway activation in platelet rich plasma (PRP) (1.5x10⁵ platelets/µL) using diluted thrombo-plastin (Dade Innovin®, 1:1000 final dilution). We studied five different LMWHs (bemiparin, enoxaparin, nadroparin, dalteparin and tinzaparin), as well as UFH at five different prophylactic and therapeutic anti-Xa final concentrations. These agents were added to control plasma from 14 healthy volunteers with equivalent anti-Xa concentrations. TG was initiated by adding the triggering solution containing CaCl₂ and the fluorogenic substrate. The analyzed TG parameters are the lag-time, the maximal concentration of thrombin (Cmax), the time to reach Cmax (Tmax), the TG velocity and the endogenous thrombin potential (ETP). Results. Bemiparin had almost no effect on TG, with concentrations below 0.60 anti-Xa IU/mL. Enoxaparin, nadroparin and dalteparin showed a similar potency in inhibiting TG at equal anti-Xa concentrations. Tinzaparin proved to be the most active LMWH in inhibiting TG and had a similar potency to UFH. Tinzaparin and UFH, with the lowest anti-Xa/anti-IIa ratio, exerted their inhibitory effect mostly by prolonging lag-time and Tmax and by reducing TG velocity, especially at concentrations below 0.40 anti-Xa IU/mL. Besides, UFH totally inhibited TG, as expressed by ETP, at a concentration over 0.40 anti-Xa IU/mL. For a given anti-Xa/anti-IIa ratio characterizing each LMWH the IC50 for each parameter was different. The IC50 for the reduction of the velocity of TG was lower as compared to the IC50 for the other parameters. (Table 1).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Bemiparin</th>
<th>Enoxaparin</th>
<th>Nadroparin</th>
<th>Dalteparin</th>
<th>Tinzaparin</th>
<th>UFH</th>
</tr>
</thead>
<tbody>
<tr>
<td>ETP</td>
<td>(&gt;1 IU/mL)</td>
<td>0.85 IU/mL</td>
<td>0.45 IU/mL</td>
<td>0.45 IU/mL</td>
<td>0.45 IU/mL</td>
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</tr>
<tr>
<td>Cmax</td>
<td>(&gt;1 IU/mL)</td>
<td>0.85 IU/mL</td>
<td>0.45 IU/mL</td>
<td>0.45 IU/mL</td>
<td>0.45 IU/mL</td>
<td>0.45 IU/mL</td>
</tr>
<tr>
<td>Velocity</td>
<td>(&gt;1 IU/mL)</td>
<td>0.85 IU/mL</td>
<td>0.45 IU/mL</td>
<td>0.45 IU/mL</td>
<td>0.45 IU/mL</td>
<td>0.45 IU/mL</td>
</tr>
</tbody>
</table>

Summary/Conclusions. Our study reinforces the concept of LMWH heterogeneity and the importance effect exerted by the additional anti-IIa activity of LMWHs, combined with their anti-Xa activity. Thus, their characteristic is to be made through their ability to inhibit TG and not only their anti-Xa/anti-IIa ratio. Furthermore, the anti-IIa inhibitory activity of heparins is primarily expressed by prolonging the lag-time and the Tmax and by reducing the TG velocity. The clinical relevance of our findings has to be studied, while the use of TG assay should be considered as a potent method to monitor anticoagulant treatment with LMWHs in the routine hematological laboratory.

0392
A GLOBAL ASSAY FOR THE ASSESSMENT OF LOW MOLECULAR WEIGHT HEPARINS ANTIITHROMBOTIC ACTIVITY IN VITRO
A. Petropoulou, G. Gerotziafas, M.M. Samama, I. Elalamy
Hotel-Dieu Hospital, PARIS, France

Background. Low molecular weight heparins (LMWHs) are derived from unfractioned heparin (UFH) by depolymerization. Thus, the potential biochemical and pharmacological differences and the ratio of the ant-Xa/anti-IIa activities varies from one product to another. LMWHs have no effect on prothrombin time and there is no a global clotting assay for the in-vitro assessment of their antithrombotic activity. Furthermore, the anti-Xa activity measurement, which is routinely used in clinical practice for monitoring the anticoagulant treatment with LMWHs, has a limited predictive value concerning the clinical outcome (thrombosis or bleeding). Aims. The aim of the present study was to assess the LMWHs global antithrombotic activity by using a rather physiological-relevant system. For this purpose we used the Thrombogram-Thrombinscope assay, a dynamic assay which describes all the phases of thrombin generation (TG) process (initiation, amplification and inhibition of TG as well as the integral amount of generated thrombin). Methods. TG was assessed after tissue factor (TF) pathway activation in platelet rich plasma (PRP) (1.5x10⁵ platelets/µL) using diluted thrombo-plastin (Dade Innovin®, 1:1000 final dilution). We studied five different LMWHs (bemiparin, enoxaparin, nadroparin, dalteparin and tinzaparin), as well as UFH at five different prophylactic and therapeutic anti-Xa final concentrations. These agents were added to control plasma from 14 healthy volunteers with equivalent anti-Xa concentrations. TG was initiated by adding the triggering solution containing CaCl₂ and the fluorogenic substrate. The analyzed TG parameters are the lag-time, the maximal concentration of thrombin (Cmax), the time to reach Cmax (Tmax), the TG velocity and the endogenous thrombin potential (ETP). Results. Bemiparin had almost no effect on TG, with concentrations below 0.60 anti-Xa IU/mL. Enoxaparin, nadroparin and dalteparin showed a similar potency in inhibiting TG at equal anti-Xa concentrations. Tinzaparin proved to be the most active LMWH in inhibiting TG and had a similar potency to UFH. Tinzaparin and UFH, with the lowest anti-Xa/anti-IIa ratio, exerted their inhibitory effect mostly by prolonging lag-time and Tmax and by reducing TG velocity, especially at concentrations below 0.40 anti-Xa IU/mL. Besides, UFH totally inhibited TG, as expressed by ETP, at a concentration over 0.40 anti-Xa IU/mL. For a given anti-Xa/anti-IIa ratio characterizing each LMWH the IC50 for each parameter was different. The IC50 for the reduction of the velocity of TG was lower as compared to the IC50 for the other parameters. (Table 1).

Table 1. The IC50% for each parameter of TG.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Bemiparin</th>
<th>Enoxaparin</th>
<th>Nadroparin</th>
<th>Dalteparin</th>
<th>Tinzaparin</th>
<th>UFH</th>
</tr>
</thead>
<tbody>
<tr>
<td>ETP</td>
<td>(&gt;1 IU/mL)</td>
<td>0.85 IU/mL</td>
<td>0.45 IU/mL</td>
<td>0.45 IU/mL</td>
<td>0.45 IU/mL</td>
<td>0.45 IU/mL</td>
</tr>
<tr>
<td>Cmax</td>
<td>(&gt;1 IU/mL)</td>
<td>0.85 IU/mL</td>
<td>0.45 IU/mL</td>
<td>0.45 IU/mL</td>
<td>0.45 IU/mL</td>
<td>0.45 IU/mL</td>
</tr>
<tr>
<td>Velocity</td>
<td>(&gt;1 IU/mL)</td>
<td>0.85 IU/mL</td>
<td>0.45 IU/mL</td>
<td>0.45 IU/mL</td>
<td>0.45 IU/mL</td>
<td>0.45 IU/mL</td>
</tr>
</tbody>
</table>

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0393
RISK OF RECURRENT VENOUS THROMBOEMBOLISM ASSOCIATED WITH PREGNANCY IN WOMEN WITH A HISTORY OF VENOUS THROMBOSIS
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Background. Previous estimates of the rate of recurrent venous thom-
boembolism (VTE) during pregnancy in women with a history of VTE have vary between 0 and 15%. Therefore, the decision to administer or withhold heparin especially in the antepartum period has been discussed controversial. In a recent study by Brill-Edwards et al. (N Engl J Med 2000;343:1439-44), no recurrences of VTE occurred in women (n=44) who had a previous episode of thrombolysis that was associated with a temporary risk factor and who also had no evidence of thrombophilia. Based on these results, antepartum heparin prophylaxis is not routinely recommended in women without thrombophilia whose previous episode of thrombosis was associated with a temporary risk factor (ACCP guidelines 2004). The aim of our study was to evaluate the risk of recurrent pregnancy-associated thrombosis in women with a history of VTE. Materials and Methods. We retrospectively studied 198 women with at least one pregnancy (275 pregnancies in total) after a one previous episode of VTE. Sixty-three women (81 pregnancies) were excluded from the analysis because of antepartum heparin prophylaxis. Results. In the subgroup of women without heparin prophylaxis (n=135), 15 (7.7%) thromboembolic events occurred antepartum in 194 pregnan-

**0395**

**SUBOPTIMAL DOSES OF LOW MOLECULAR WEIGHT HEPARIN IN THE TREATMENT OF VENOUS THROMBOEMBOLIC DISEASE**

M.J. Bruscas Alijarde, J.A. Nieto Rodríguez, M.D. Ruiz Ribó, E. Grau Segura, R. Lecumberri, A. Grau Martín, E. Rague Sanz, R. Guijarro Merino

Hospital Virgen de la Luz, CUENCA, Spain; Hospital de Navarra, PAMPLONA, Spain; Hospital de Figueres, GERONA, Spain; Hospital de Terrassa, BARCELONA, Spain; Hospital Carlos Haya, MALAGA, Spain

Background. A number of patients with venous thromboembolism (VTE) are treated with suboptimal doses of low molecular weight heparin (LMWH). However, there are no clinical trials that have established the efficacy of these doses. Aims. The objective of this study was to evaluate the evolution of patients treated with suboptimal LMWH (60-149 UI/Kg/d) as compared with patients treated with standard doses (150 UI/Kg/d). Methods. Analysis of data from a prospective, multicentre registry of VTE (RIETE) entering consecutive patients with VTE diagnosed by objective tests. Patient characteristics, antithrombotic treatments and 3-month outcomes were recorded. Results. Up to July 2005, 10,524 were diagnosed with deep vein thrombosis (DVT) or pulmonary embolism (PE) and treated initially with LMWH; 1,547 (14.7%) patients received suboptimal LMWH (mean, 122 UI/Kg/d) and 9,977 (85.3%) patients received full-dose LMWH (mean, 192 UI/Kg/d). Suboptimal doses of LMWH were significantly (p<0.05) associated with:

- Younger patients (63.3 vs. 65.7 years), outpatient treatment (26.7% vs. 12.5%), use of vena cava filters (2.4% vs. 1.2%), recent episodes of major bleeding (4.5% vs. 1.6%) and renal failure (15.6% vs. 13%).
- Standard doses of LMWH were more frequently used in patients with proximal DVT (82.6% vs. 78%), PE (42.5% vs. 28.8%). At the end of the follow-up, there were no significant differences in the rates of mortality (7.7% vs. 7.8%), VTE recurrence (2.7% vs. 2.3%), or fatal haemorrhage (3.2% vs. 2.6%) between the suboptimal and the standard group.
- Neither were there any differences in the subgroup of patients with PE (mortality, 10.1% vs. 9.7%; recurrence, 2.7% vs. 2.5%; fatal haemorrhage, 0.9% vs. 0.6%) or in the whole group after a 2-week follow-up (mortality, 2.7% vs. 2.9%; recurrence, 0.8% vs. 0.8%; fatal haemorrhage, 0.3% vs. 0.3%).
- During the first two weeks, major bleeding rate was significantly higher in the suboptimal LMWH group (2.0 vs. 1.1%; p=0.05).

**0394**

**MAJOR HAEMORRHAGE BEFORE VENOUS THROMBOEMBOLISM: DIFFERENT OUTCOMES DEPENDING ON THE BLEEDING SITE**

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Hospital Virgen de la Luz, CUENCA, Spain; Hospital Lluís Alcanyís, VALENCIA, Spain; Clínica Universitaria de Navarra, PAMPLONA, Spain; Hospital de Figueres, GERONA, Spain; Hospital de Terrassa, BARCELONA, Spain; Hospital Carlos Haya, MALAGA, Spain

Background. Patients with a recent episode of major bleeding are usu-

ally excluded from clinical trials. The management of these patients is not evidence based and their outcomes are unknown. Aims. To study outcomes of patients with VTE and a recent episode (<30 days) of major bleeding before VTE diagnosis, according to the bleeding site and the time interval between bleeding and VTE. Methods. Analysis of the data from a prospective, multicentre registry of VTE (RIETE) entering consecu-

tive patients with VTE diagnosed by objective tests. Patient character-

istics, antithrombotic treatments and 3-month outcomes were recorded. Results. Of the 12,302 patients enrolled up to July 2005, 306 (2.5%) patients had had a recent episode of major bleeding, 106 (36%) gastrointestinal, 94 (30%) intracranial, and 96 (32%) from other sites. When compared with the group of patients without recent haemorrhage, the mortality rate (14.1% vs. 8.0%), major haemorrhage rate (6.2% vs. 2.3%) and fatal haemorrhage rate (2.6% vs. 0.5%) were significantly higher (p<0.01) in the recent bleeding group. With the exception of the intracranial site, previous bleeding patients had an increased risk of new bleeding (CI 95%: 1.4-4.9) and fatal bleeding (CI 95%: 2.2-9.3) from other sites. Other HR 1.9, 95% IC: 1.2-3.1. Other HR 2.0, 95% CI:1.2-3.3). Episodes of major bleeding were associated with previous GI haemorrhage (HR 2.8, 95% IC: 1.4-5.3). A time interval of less than 2 weeks between major bleeding and VTE diagnosis was also associated with an increased risk of new episodes of bleeding in 194 pregnancies (CI 95%: 1.4-4.9). IC: 2.4-9.5. Occurred major bleeding (HR 2.4, 95%CI: 1.2-5.0) and death (HR 2.7, 95% IC: 1.7-4.1). Conclusion. The risk of new bleeding episodes or death during a 3-month follow-up was increased in patients with VTE and 1) previous bleeding other than intracranial and 2) bleeding episodes occurring less than two weeks before VTE diagnosis. The antecedent of recent intracranial bleeding identifies a subgroup of patients with VTE and a better prog-

**0396**

**ELEVATED PROTHROMBIN FRAGMENT F1+2 LEVELS DURING PREGNANCY IN WOMEN WITH PREVIOUS VENOUS THROMBOEMBOLISM**

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Universitätsklinikum Düsseldorf, DUESSELDORF, Germany; Universitätsklinikum Düsseldorf, DUESSELDORF, Germany

Background. Changes in blood coagulation and fibrinolysis during pregnancy create a state of hypercoagulability. This phenomenon predisposes to venous thromboembolism. Women with prior venous thromboembolism are believed to have a higher risk of venous thromboembolism in a subsequent pregnancy. The risk is higher if the past episode was unprovoked, and the risk is higher if the past episode was associated with biochemical abnormalities such as factor V Leiden G1691A (factor V Leiden). Since the positive predictive value of factor V Leiden and other thrombophilic risk factors for pregnancy associated thrombosis is low, additional indicators of hypercoagulability are needed. Indicators of hypercoagulability in normal pregnancy are increased levels of prothrombin fragment 1+2. Aim. We hypothesized that women with factor V Leiden or a previous venous thromboembolism are at a higher hypercoagulable state during subsequent pregnancies than women without prior thrombotic complications or without factor V Leiden. Methods. In a prospective study, we determined prothrombin fragment F1+2
over pregnancy among 109 women (175 measurements) with previous venous thromboembolism, and among 185 pregnant women (75 measurements) without previous venous thromboembolism. The prothrombin fragment F1+2 levels were statistically analyzed over time using a mixed model. This model allows a longitudinal analysis of the influence of a between-subjects factor (e.g. history of thrombosis) on prothrombin fragment F1+2 levels, the influence of a within-subjects factor (weeks of gestation) on prothrombin fragment F1+2 levels, and the interaction of the history of thrombosis and weeks of gestation representing a change of risk factor-dependent differences over time (weeks of gestation). Results. Among women with a previous history of venous thrombosis, prothrombin fragment F1+2 values were significantly higher during the course of pregnancy than among pregnant women without venous thrombosis (p=0.001). The results were adjusted for the physiological increase of prothrombin fragment F1+2 over pregnancy and independent from heparin prophylaxis. In addition, factor V Leiden was independently associated with increased levels of prothrombin fragment F1+2 (p=0.05). Conclusion. Thus, determination of indicators of hypercoagulation like prothrombin fragment F1+2 represent an additional approach independent from known and unknown risk determinants of thrombosis to identify women at risk for venous thromboembolism during pregnancy.

0397
RECURRENT FETAL LOSS: PROSPECTIVE EVALUATION OF THE EFFICACY OF THREE DIFFERENT THROMBOPROPHYLAXIS REGIMENS: ASPIRIN VERSUS LOW MOLECULAR WEIGHT HEPARIN VERSUS LOW MOLECULAR WEIGHT HEPARIN PLUS ASPIRIN
A. Chistolini,1 F. Torelli,2 A. Giancotti,2 P. Pignoloni,2 B. Mutto,1 C. Cosimo,1 C. Santoro,2 M.G. Mazzucconi1
1University La Sapienza Rome, ROME, Italy; 2University La Sapienza, ROMA, Italy

Background. Growing evidence suggests that thrombophilia is associated with an adverse pregnancy outcome, but there is a lack of controlled trials of antithrombotic prophylaxis to prevent pregnancy complications. Aims. The aim of our study was the prospective evaluation of the efficacy of three different thromboprophylaxis regimens in women with two or more unexplained pregnancy losses. Methods. A total of 361 women with pregnancy loss were studied for thrombophilia: 226 (72.6%) did not present thrombophilia; 99 (27.4%) were positive for one or more thrombophilic parameters. 94/361 women got pregnant (none of these patients presented APA syndrome); 56 (59.6%) with negative congenital thrombophilic screening, 38 (40.4%) with positive congenital thrombophilic screening. 94 patients were randomly assigned to one of the three thromboprophylaxis regimens from the 8th week of pregnancy: low dose aspirin 100 mg daily (arm A), enoxaparin 40 mg daily (arm B), aspirin 100 mg plus enoxaparin 40 mg daily (arm C). All patients with thrombophilia were treated with implan ted enoxaparin 40 mg once per week at 36th week of pregnancy and the 6th week after delivery. Results. Thromboprophylaxis therapy was associated with 73 (77.7%) live births and 21 (23.3%) pregnancy losses: in a total of 305 previous pregnancies, we observed 57/305 (12.1%) live births and 268/305 (87.9%) pregnancy loss (p=0.0001). In the 56 patients with negative thrombophilia screening, thromboprophylaxis was associated with 49 (87.5%) live births and 7 (12.5%) pregnancy losses: in a total of 150 previous pregnancies, these negative patients had 18/150 (12%) live births and 132/150 (88%) pregnancy losses (p=0.0001). Considering the three different therapeutic regimens, we noted in arm A: 14/19 (73.7%) live births and 5/19 (26.3%) pregnancy losses; arm B: 16/18 (88.9%) live births and 2/18 (11.1%) pregnancy losses; arm C: 19/19 (100%) live births and 0/19 (0%) pregnancy losses. In the 38 patients with positive thrombophilia screening, the thromboprophylaxis was associated with 24 (63.2%) live births and 14 (36.8%) pregnancy losses: in a total of 155 pregnancies, these positive patients had 19/155 (12.2%) live births and 136/155 (87.8%) pregnancy losses (p=0.0001). In these patients, considering the three different therapeutic regimens, we noted in arm A: 3/12 (25%) live births, and 9/12 (75%) pregnancy losses; in arm B: 10/13 (76.9%) live births and 3/13 (23.1%) pregnancy losses; in arm C: 11/13 (84.6%) live births and 2/13 (15.4%) pregnancy losses. Conclusions. Our study shows that thromboprophylaxis therapy is effective in women with recurrent pregnancy losses. In the negative thrombophilic patients, thromboprophylaxis is effective: (87.5%) live births. In these patients, no difference was found in the three therapeutic regimens. In the positive thrombophilic patients, therapy with enoxaparin or aspirin plus enoxaparin was more effective than aspirin treatment (p=0.0169 and p=0.0048, respectively). No difference was found between enoxaparin versus aspirin plus enoxaparin.

0398
THE RISK OF RECURRENT VENOUS THROMBOEMBOLISM IN PREGNANCY AND PÆRPERIUM WITH NO ANTITHROMBOTIC PROPHYLAXIS
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1Catholic University, ROME, Italy; 2A. Bianchi Bonomi Center, UNIVERSITY OF MILAN, Italy; 3Inst. Hematology, Catholic University, ROME, Italy

Background. Whether or not pregnant women with a previous venous thromboembolism (VTE) should receive antithrombotic prophylaxis is a matter of debate. Aims. To estimate the probability of recurrent VTE during pregnancy and puerperium in women stratified according to the presence of inherited thrombophilia and/or the circumstances of the first VTE. Methods. We studied a retrospective cohort of 1401 women with a first VTE occurred before 40 years of age and referred to our centers for laboratory evaluation. The previous clinical history was taken at the admission blinded to the laboratory results. All the events were objectively diagnosed. Inherited thrombophilia was defined as the presence of deficiency of antithrombin, protein C, and protein S. Factor V Leiden, prothrombin G20210A. Women with antiphospholipid antibodies were preliminarily excluded from the study. After the first VTE 197 women were pregnant at least once. Further exclusion criteria of women were a history of recurrence between the first thrombosis and pregnancy (none of these patients presented APA syndrome): 56 (59.6%) with negative congenital thrombophilic screening, 38 (40.4%) with positive congenital thrombophilic screening. Among women with a previous history of venous thrombosis, prothrombin fragment F1+2 values were significantly higher (77.7%) live births and 21 (23.3%) pregnancy losses: in a total of 305 previous pregnancies, we observed 57/305 (12.1%) live births and 268/305 (87.9%) pregnancy loss (p=0.001). The results were adjusted for the presence of deficiency of antithrombin, protein C, and protein S, factor V Leiden, prothrombin G20210A. Women with antiphospholipid antibodies were preliminarily excluded from the study. After the first VTE 197 women were pregnant at least once. Further exclusion criteria of women were a history of recurrence between the first thrombosis and pregnancy (none of these patients presented APA syndrome): 56 (59.6%) with negative congenital thrombophilic screening, 38 (40.4%) with positive congenital thrombophilic screening. Among women with a previous history of venous thrombosis, prothrombin fragment F1+2 values were significantly higher during the course of pregnancy than among pregnant women without venous thrombosis (p=0.001). The results were adjusted for the physiological increase of prothrombin fragment F1+2 over pregnancy and independent from heparin prophylaxis. In addition, factor V Leiden was independently associated with increased levels of prothrombin fragment F1+2 (p=0.05). Conclusion. Thus, determination of indicators of hypercoagulation like prothrombin fragment F1+2 represent an additional approach independent from known and unknown risk determinants of thrombosis to identify women at risk for venous thromboembolism during pregnancy.

0399
THROMBOELASTOGRAPHIC STUDY IN whole BLOOD EMBOLING A FIBRIN POLYMERIZATION INHIBITOR (PFBABLOC7) AND AN INHIBITOR OF ACTIN POLYMERIZATION (CYTOCHALASIN D)
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1Hotel-Dieu Hospital, PARIS, France; 2Institut Pasteur, PARIS, France

Background. Minimal tissue factor (TF) triggered whole blood thromboelastography (TEG) provides a valuable tool for studying the kinetics of thrombus formation (expressed by the parameters R, k and λ-angle) and the physical characteristics of the thrombus, such as its firmness and the elastic modulus shear expressed by the parameters of maximal amplitude (MA) and the G respectively. Aims. We studied the influence of fibrin polymerization and platelet functional status on the thromboelastographic trace after minimal TF pathway activation in whole blood using increasing concentrations of a fibrin polymerization inhibitor (Gly-Pro-Ang-Pro-OH, AcOH; Pefabloc-FG) and an inhibitor of actin polymerization (Cytochalasin D). Methods. Coagulation was triggered in a plastic disposable cup containing 20 µl CaCl2 (0.2M) and 10 µl of diluted thromboplastin by the addition of 380 μl whole blood, supplemented with Pefabloc-FG or Cytochalasin D. Data acquisition was done during
demonstrates the close association between the quality of the thrombus and the strength and elastic modulus of the thrombus. Results. Pefabloc-FG at concentrations higher than 5 mg/mL prolonged the R and k-times and decreased the α-angle in a concentration-dependent manner but it did not modify MA and G. Pefabloc-FG at 5 mg/mL, completely inhibited thrombus formation. Cytochalasin D did not modify R-time but decreased the α-angle, MA and G. The effect of cytochalasin D was pronounced on MA and G. A combination of Pefabloc-FG (0.5 mg/mL) and cytochalasin D (50 μM) significantly decreased α-angle compared to control as well as their single effect. However, G was dramatically reduced in the presence of cytochalasin D, without any additional effect by Pefabloc-FG. Conclusions. This study confirms the importance of fibrin polymerization on the kinetics of thrombus formation and demonstrates the close association between the quality of the thrombus and the functional status of platelets. Normal platelet contractile forces are of major importance for the maximum amplitude of TEG which is related to the strength and elastic modulus of the thrombus.

PATIENTS WITH ANTIPHOSPHOLIPID ANTIBODIES HAVE A HIGH INCIDENCE OF ANTI-ADAMTS13

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Background. Thrombotic thrombocytopenic purpura (TTP) and antiphospholipid syndrome (APS) are autoimmune diseases associated with thrombosis. TTP is associated with thrombocytopenia, microangiopathy, variable multi-organ ischaemia and reduced ADAMTS13 activity. The mechanism of thrombosis in APS is unclear, but catastrophic cases of APS can result in similar microangiopathic features to those of TTP. Aim. Autoantibodies that neutralise ADAMTS13 are commonly found in patients with acquired idiopathic TTP, but their incidence in other thrombotic microangiopathy is not well investigated. Method. In our ongoing study we have currently assessed 76 patients with antiphospholipid antibodies (66/76 primary APS, 8/76 SLE, 2/76 other autoimmune diseases. The pathophysiological mechanism that explains this association is still to be determined. Aims. This study was designed in order to evaluate the correlation between homocysteine and markers of endothelial function and to evaluate the effect of vitamin supplementation on these markers in patients with VTE. Methods. This study was a multicentre, randomized, double-blind, placebo-controlled trial. We randomized 105 patients with a first event of objectively confirmed VTE, aged between 18 and 70 years, to receive either vitamin supplementation (follic acid 5 mg, vitamin B6 50 mg and vitamin B12 0.4 mg) or placebo. Blood was collected at randomization and 8 weeks after the intervention period. Results. Ninety-nine (94.3%) completed the 8-week period of treatment. We compared patients with basal homocysteine above the highest tertile (12.6 micromol/L) with those below the lowest tertile (9.9 micromol/L) and observed a difference in the plasma plasminogen activator inhibitor type1 (PAI-1), Factor VIII-C and von Willebrand factor antigen (vWF) between the two groups. Vitamin supplementation decreased the homocysteine median levels from 10.7 to 8.1 micromol/L (29% reduction). There was a significant increase in the levels of tissue plasminogen activator (t-PA) from 6.1 to 9.0 mmol/L in the group treated with vitamins, (p=0.0008, Wilcoxon rank-sum test) and also in the group treated with placebo, although less evident (from 8.0 to 9.5 mmol/L, p=0.08). PAI-1 levels did not change after 8 weeks both in the vitamin and in the placebo groups. Both t-PA and PAI-1 levels significantly increased only in the group of patients above the highest tertile of homocysteine who received vitamin supplementation (p=0.0004 and p=0.014, respectively). There was no change in the levels of these two markers in patients with homocysteine levels below the lowest tertile or in patients who received placebo with higher and lower homocysteine levels. vWF and factor VIII-C were unaffected by both vitamins and placebo, even in patients above the highest tertile of homocysteine. Conclusions. In patients with VTE, homocysteine reduction by B-vitamin supplementation caused a significant increase on t-PA. In patients with higher levels of homocysteine both t-PA and PAI-1 were increased by vitamins, although the basal levels of both markers were similar to the patients with lower levels.

D-DIMER LEVEL IS ASSOCIATED WITH THE SEVERITY OF PULMONARY EMBOLISM

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Background. PE is a potentially fatal condition with a 3-month mortality rate reaching 15%. D-dimer is widely used as an initial test in the work-up of suspected PE. Risk factors like clinical presentation, right ventricular dysfunction and elevated cardiac biomarkers are associated with adverse prognosis and poor outcome. Patients carrying such risk factors may require more intensive treatment and usually benefit from thrombolysis. Aim. To investigate the association between the level of D-dimer and the most proximal level of PE. Methods. From Feb 2002 to Dec 2003, 99 consecutive patients were diagnosed with PE using 4-detector row CT angiography. The patients were equally divided between the upper and lower level of PE. Results. Of the 96 patients who received thrombolysis. In the subgroup of patients with D-Dimer in the upper quartile, 8 patients (33%) received thrombolysis, compared to 4 in the intermediate and none with low D-dimer. There were no in-hospital
deaths, and the 3-month mortality rate in this cohort was 4%. Summary of statistical analysis showed that the level of D-dimer was related to the severity of PE assessed by various radiological, biochemical and clinical markers. Hence D-dimer could be of value as prognostic marker for the severity of PE. However, the low mortality rate precludes us from making conclusions regarding the predictive value of D-dimer on mortality. The prognostic value of D-dimer and its clinical significance need to be evaluated in properly designed prospective studies.

**Table 1.**

<table>
<thead>
<tr>
<th>D-dimer mg/L</th>
<th>Low 0.5-1.8</th>
<th>Intermed. 1.9-12.1</th>
<th>High 12.2-20</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N=26</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAO&gt;40%*(OR)</td>
<td>1(1)</td>
<td>20(17)</td>
<td>18(64)</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>RV/LV ratio&gt;1.9*(OR)</td>
<td>2(1)</td>
<td>21(9)</td>
<td>16(21)</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>TNT&gt;0.01 mg/ml*(OR)</td>
<td>1(1)</td>
<td>12(10)</td>
<td>12(34)</td>
<td>&lt;0.0005</td>
</tr>
</tbody>
</table>

* TNT was measured in 67 patients; 18 in Low, 31 in intermediate and 18 in the high D-dimer category.

**0404**

**THE VALUE OF THE DETERMINATION OF ACTIVATED PROTEIN C RESISTANCE (APC-R) IN HEMODIALYSIS PATIENTS**

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**Background.** The genetic mutation of factor V Leiden which is characterized by an increased resistance to activated protein C (APC-R) is one of the most common inherited thrombophilia factors. Several studies suggest that about 4-8% of the general population is heterozygous for factor V Leiden. These rates are higher in some populations such as the northern Europeans. In Greece and Sweden some studies showed increased rates above 10%. Non molecular laboratory tests can demonstrate high sensitivity (99.6%) and specificity (99.7%) the presence of this mutation. These tests can be performed in many coagulation analyzers and with low cost. On the other hand fistula or graft thrombosis is a common and costly complication in hemodialysis patients. Recent studies suggest that thrombophilia is associated with access thrombosis in these patients. Aim. The aim of this study was to establish the value of APC-R determination in hemodialysis patients by accessing the association between increased APC-R and access thrombosis. Methods. In this retrospective study, 75 patients (36 men, mean age 63.2±10.9y and 39 women, mean age 62±12.2y) were selected from the hemodialysis Unit of the University Hospital, Iraklion Greece, between July 2003 and March 2005. The mean time on hemodialysis was 74.8±43.1 months. All patients were tested for antithrombin III, protein S, protein C, activated protein C resistance (APC-R), Lupus anticoagulant, antiphospholipid antibodies (panel), factors VIII and XI, homocysteine and lipoprotein(a). All participants were divided into two groups, those with access thrombosis (42 patients) and those with no access thrombosis (33 patients) and we assessed the prevalence of each thrombophilia factor to be statistically significant. Results. Statistical analysis showed that among all tested thrombophilia factors only the presence of APC-R had a statistically significant association with thrombosis. Overall, nine patients (12%) had an increased resistance to activated protein C. All these patients had at least one episode of access thrombosis (100%). Univariate analysis to estimate crude (unadjusted) odds ratio showed a 2 times higher risk for access thrombosis in these patients: X²=7.862, df=1, p=0.005, OR 1.97 (95%CI: 1.56-2.49). In the previous statistic model no statistically significant differences were found after adjusting for sex, age, smoking habits, months in hemodialysis Hypertension, Diabetes Mellitus, Coronary Artery Disease, Cerebrovascular Disease, Peripheral Arterial Disease and Malignancy. Conclusion. This study revealed a statistically significant association between access thrombosis and increased APC-R in hemodialysis patients. This indicates that the determination of APC-R should be considered in these patients especially in populations with a high prevalence of factor V Leiden.

**0405**

**ALTERATIONS OF HEMOSTASIS AFTER LAPAROSCOPIC AND OPEN SURGERY**

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**Background.** After tissue injury because of trauma or surgery, alterations of hemostasis are observed and there is a risk for postoperative thromboembolic complications. Laparoscopic surgery, by causing limited tissue injury, seems to be associated with a lower risk for thromboembolic than open surgery. However, recent studies are conclusive as it is based on a few studies, most often non-randomized. Aim. This prospective randomized study was conducted in order to detect potentially existing differences in activation of coagulation and fibrinolytic pathways between open and laparoscopic surgery. Methods. From January to September 2005 40 patients ASA1 and ASA2 were randomly assigned to undergo laparoscopic (group A, n=20) or open cholecystectomy (group B n=20) by the same surgical and anesthesiology team. Demographic data were comparable. Blood samples were taken a) preoperatively, b) at the end of the procedure, c) 24 h postoperative.
ly and d) 72 hrs postoperatively. The following parameters were measured: platelets, soluble fibrin monomer complex (F5-test), fibrin degradation products (FDP), D-Dimers (D-D), fibrinogen (FB), activated partial thromboplastine time (APTT), prothrombin time (FT). Thrombin-antithrombin III complexes (TAT) were measured at 24 hrs and 72 hrs postoperatively. Prothrombin fragment 1+2(F1+2) was measured at 24 hrs and 72 hrs postoperatively in 11 patients of group A and 13 patients of group B respectively. Results. Preoperatively, values of all haemostatic parameters were within normal limits in both groups. Immediately postoperatively, values of the coagulation markers TAT and F1+2 were significantly increased in the open surgery group as compared to the laparoscopic surgery group (p<0.05). Values of marker D-Dimers were also significantly increased in the open surgery group (p<0.01) immediately postoperatively and remained like that throughout the whole period of observation. Values of the coagulation marker FIB decreased slightly in both groups at 24 hrs postoperatively but there was a significant increase in the open surgery group as compared to the laparoscopic group (p<0.01) which remained like that thereafter. The APTT and PT values began to rise slightly in both groups but there was not observed a significant difference at any time between the two groups. The coagulation marker F.S. test became positive twice in the open surgery group starting immediately postoperatively and only once at 72hrs postoperatively in the laparoscopic group. Concentration of the fibrinolysis marker β2GPI in the open surgery group starting immediately postoperatively and this difference became significant 72 hrs postoperatively (p<0.05). No patient from either group suffered thromboembolism or abnormal bleeding as a post-operative complication. Conclusions. Open surgery as compared to laparoscopic procedure leads in activation of the clotting system of a higher degree than in laparoscopic surgery group implying thus a greater thromboembolic risk for patients undergoing open surgery. Subclinical fibrinolysis is also more profound at the open surgery group. Although of a lower degree, hypercoagulability is still observed in patients undergoing laparoscopic surgery and therefore routine thromboembolic prophylaxis should be considered.

0406
ROLE OF THE V617F MUTATION OF THE JAK2 IN PATIENTS WITH THROMBOSIS
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Background. Polycythemia Vera (PV) and Essential Thrombocytemia (ET) are Chronic Myeloproliferative Diseases (MPD) characterized by increased cell counts.

A single point mutation of JAK2 (Val617Phe) has been detected in most PV and in half the patients with ET. On the other hand, many patients suffer from thrombosis without an underlying cause. However, an underlying MPD has been demonstrated especially in patients with thrombosis in uncommon locations, such as Budd-Chiari syndrome. The diagnosis of this underlying MPD is often very difficult and requires sophisticated methodologies. Before the advent of the JAK2 mutation, X-chromosome inactivation patterns and in vitro erythroid colony formation have been used. These methodologies are cumbersome and its use is restricted to some laboratories, but the investigation of the single point mutation (Val617Phe) of JAK2 is now readily available. Aims. The presence of the JAK2 mutation was assessed in a cohort of patients with thrombosis. Methods. A cohort of 309 patients with thrombosis were recruited from November 1997. Their DNA samples were analyzed by the allele-specific PCR methodology (1). DNA samples from 25 patients with PV and 7 with ET were selected as positive controls. Results. In PV and ET, 24 out of 25 cases showed the JAK2 mutation (96%). As for the patients with thrombosis, 1 out of the 309 patients with thrombosis was positive. This case was a 69-years-old male with 3 episodes of deep venous thrombosis and two of superficial venous thrombosis. His thrombophilia study was negative. This patient has been controlled in our department since 1997 and his Hb ranged from 160-168 g/l. Platelets and leucocytes were always normal. Conclusions. An obscure MPD is a very improbable cause of thrombosis. The investigation of the mutation V617F of the JAK2 gene should be reserved for special cases, such as patients with thrombosis in uncommon localization or patients with increased cell counts.

Reference

0407
EVALUATION OF NEW COMMERCIAL ELISA KITS IN THE LABORATORY DIAGNOSIS OF ANTIPHOSPHOLIPID SYNDROME IN VIEW OF THE REVISED CLASSIFICATION CRITERIA OF THE ANTIPHOSPHOLIPID SYNDROME
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Ghent University Hospital, GHENT, Belgium

Since the publication of the 1999 Sapporo criteria for the classification of the antiphospholipid syndrome (APS) new clinical and laboratory insights have led to a recent update. These revised criteria now include testing for the presence of IgG and IgM β2-glycoprotein I (β2GPI) with a positive titer being defined as higher than the 99th percentile of the normal population. aCL continues to be measured by a standardised ELISA. aCL positivity continues to be detected according to the ISTH guidelines. We have evaluated a newly developed Asserachrom® Anti-phospholipid antibodies immunosassay line (Diagnostica Stago, Asnières, France) for the detection of antiphospholipid antibodies (APA) in a lupus anticoagulant (LAC) positive (n=157) and a LAC negative (n=134) population. The Asserachrom® APA Screen has been proposed to be used as a first screening assay for the qualitative detection of APA. Positive samples can be further investigated by the Asserachrom® APA IgG,M to determine the isotype and the quantitative antibody level. As 0 to 10% of the APS patients are only positive for anti-β2GPI (Mayak S, J Thromb Haemost, 2006) the Asserachrom® anti-β2GPI IgG and Asserachrom® anti-β2GPI IgM have also been proposed to be used in parallel to the Asserachrom® APA Screen. Despite that anti-prothrombin antibodies (αPT) are not included in the updated laboratory criteria they have been tested in this evaluation (Asserachrom® anti-prothrombin IgG,M). This new line of ELISAs uses monoclonal antibody based standardisation in accordance with the recommendations of the Standardisation Group of the European Forum on Antiphospholipid Antibodies for the APA, β2GPI assays. Imprecision characteristics performed with the included control material for all ELISAs were good, with coefficient of variation (CV) ranging from 4.9% to 13.9%. Cut-off values calculated with 99th percentile, as advised by the updated laboratory criteria, are higher than those currently proposed by the manufacturer (calculated with 97.5th percentile). The Asserachrom® APA Screen showed 2.6% false positive and 0.7% false negative results when compared with the Asserachrom® APA IgG,M which is acceptable. 49 patients out of 271 (18.1%) were positive for β2GPI antibodies. For 23 patients out of those 49, the Asserachrom® APA Screen was negative. This is in agreement with the above observation that the anti-β2GPI may be the only test positive (Mayak S., J Thromb Haemost, 2006). 20 patients out of 271 (7.4%) had a positive titer for αPT antibodies, 40.0% of them (8/20) were negative with the Asserachrom® APA Screen. In conclusion, the Asserachrom® antiphospholipid antibodies line shows good performance characteristics and is a practical tool in the laboratory diagnosis of the APS. Own cut-off values should be calculated for each laboratory with the 99th percentile. As the anti-β2GPI may be the only test positive, the Asserachrom® APA Screen, the Asserachrom® anti-β2GPI IgG and the Asserachrom® anti-β2GPI IgM should be performed in parallel.

0408
DIFFERENTIAL EXPRESSION AND REGULATION OF PROTEASE RECEPTORS IN MONOCYTES FROM PATIENTS WITH ANTIPHOSPHOLIPID SYNDROME.
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1Hospital Reina Sofia, CORDOBA, Spain; 2St. Thomas Hospital, LONDRES, United Kingdom; 3Hospital ‘Reina Sofia’, CORDOBA, Spain

Background. Patients with antiphospholipid antibodies (aPL) have an increased expression of Tissue Factor (TF) on monocytes and this can be one of the mechanisms leading to thrombosis. Depending on the cell type and the biological settings, TF seems to affect cellular properties and PAR(s) functions in specific cellular processes remain unclear. In addition to its anti-inflammatory and immunosuppressive properties, statins have been shown antithrombotic effect, although the molecular mechanisms leading to this effect are not yet fully understood. Objectives. To investigate i) the aberrant expression of PARs in monocytes of patients with antiphospholipid syndrome (APS), and ii) the in vivo effects
of Fluvastatin on both PARs and TF expression in this experimental setting. Methods. Ten patients with APS and previous history of thrombosis received Fluvastatin (40 mg/day) for one month. Blood samples were obtained before treatment and after one and three month of treatment. Monocytes were isolated from peripheral blood mononuclear cells by magnetic depletion of non-monocytes. TF and PAR expressions at both mRNA and protein levels were measured by real-time RT-PCR, western blot, and flow cytometry. Results. Analysis of mRNA of the four PAR described to date in humans (PAR-1 to PAR-4) revealed that PAR1 was de most abundant member of the PAR family in the monocytes of APS patients. Significantly increased expression of PAR2 was also observed in relation to the control group. PAR3 expression was also demonstrated, but not significantly altered versus healthy controls. PAR4 expression was absent. Monocytes from all the APS patients studied showed significant inhibition of TF expression at both mRNA and protein levels after one month of Fluvastatin treatment (p<0.002). These levels then suffered a slowly recovery, although remained significantly lower than control values after three months of the end of the treatment. Interestingly, mRNA expression levels of PAR2 strictly paralleled this behavior in response to fluvastatin treatment. Conclusion. These results provide the first demonstration of increased PAR expression in monocytes from APS patients. Statins drugs indirectly downregulate thrombin generation at the cellular levels. Our study for explaining the anti-thrombotic properties of statins couples the downregulation of TF with inhibition of PAR expression. Thus, PAR blockade might also draws increasing attentions to its therapeutic applications for anti-thrombosis.

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0409
PROCOAGULANT FACTORS IN PATIENTS WITH CANCER
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Background. Clotting activation and thromboembolic manifestations are common features in patients with cancer. Tumor cells can directly activate the clotting through two procoagulants: tissue factor (TF) and cancer procoagulant (CP). Aims: the aim was to evaluate the levels of the TF and CP in patients with different tumors in order to: 1) to establish an association between these markers and tumor localization, 2) to establish a correlation between the levels of procoagulants and status of disease, 3) to evaluate if the treatment with chemotherapy induced some modifications on the levels of procoagulants, 4) to evaluate the possibility of using procoagulants as predictor factors in the development of thrombosis. Methods. Sixty-one patients with different types of cancer (lung, breast, digestive and genitourinary) and 20 normal controls were included. The activity of TF and CP was studied in blood serum. Statistical analysis of the data was performed by the two-tailed Fisher exact test. Results. The TF was increased in 72.5% and 0% (p<0.01) of cancer patients and normal controls, respectively. The FC was demonstrated increased in 88% of the cancer patients but in healthy controls it was increased in only 15% (p<0.01). The patients with genitourinary cancer presented the highest values of both procoagulants coinciding with a major prevalence of thrombotic events. The activity CP was found in 95% of patients with stages I and II but in patients with stages III and IV disease it was found in 65% (ns). They were not difference in levels of both procoagulants between the patients treated with chemotherapy and those with other treatments. Conclusions. TF and CP are elevated in patients with cancer. The highest values of both procoagulants are in the genitourinary cancer group in agreement with the greater presence of thrombosis observed in this group. A clinical follow up would be an important aspect to have a more clear idea on the potential value of these procoagulants and the tendency to develop thrombosis in patients with cancer.

0410
THROMBOPHILIC RISK FACTOR OF C46T POLYMORPHISM IN THE FACTOR XII GENE FOR VENOUS THROMBOSIS
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Background. Currently the dates in relation to thrombophilic risk factor of C46T polymorphism in the factor XII gene are contradictory; Tira-do et al. are suggesting that the polymorphism itself is an independent risk factor for venous thromboembolism, another hand, Bertina et al., in their study, are showing similar results for the frequencies of the C46T genotypes in patients and controls. Aims. The objective of this study is to define the prevalence of C46T polymorphism in the factor XII gene in human blood donors and patients with venous thromboembolism and to establish his thrombophilic risk factor. Methods. A prospective study case/control we were included 516 subjects (219 patients and 297 controls). The patients with venous thromboembolism are diagnosed: 161 Deep Venous Thrombosis, 34 Pulmonary Embolism and 24 with both disease. The controls are healthy persons, blood donors, they were included in study voluntarily. The sex and age of the patients and the controls have a similar distribution. The detection of polymorphism factor XII 46C/T by PCR in real time, in liquid phase, in a LightCycler (Roche diagnostics) thermal cycle was made. The sequences of the allele primers are, forward: TTCTTCTgCTTCCAgTCCC and reverse: ATggCTCATggCaTgAATA. Stastical methodology, the descriptive was made by groups in patients and controls; to estimate the risk by square-chi proof. Results. The results of prevalence of C46T polymorphism Factor XII gene in patients and controls are: patients CC 132 (60.3%), CT 75 (34.2%), TT 12 (5.5%) controls CC 200 (67.3%), CT 92 (31.0%), TT 5 (1.7%); in the next table are showed. The estimate risk to have got a venous thromboembolism event in relation to genotype TT of C46T polymorphism factor XII gene, with CI 95% is 3.6 (1.2;10.56) p=0.012. Conclusion. The allele homozgyous T of C46T polymorphism in the factor XII gene is a thrombophilic risk factor for venous thromboembolic disease

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Table 1.

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<th>Total</th>
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<td>132 (60.3%)</td>
<td>75 (34.2%)</td>
<td>12 (5.5%)</td>
</tr>
<tr>
<td>Controls</td>
<td>297</td>
<td>200 (67.3%)</td>
<td>92 (31.0%)</td>
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THE DIVERSE IMPACT OF SUBMAXIMAL EXERCISE IN THE FIBRINOGENIC, COAGULATION AND INFLAMMATORY MECHANISMS IN PATIENTS NEVER TREATED FOR THEIR HYPERTENSION

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Background. Hypertensive patients are known for their atherogenetic background, based on haemostatic and inflammatory disorders, as well as for endothelial dysfunction. It is also known that exercise decreases cardiovascular risk in healthy individuals and in patients with coronary heart disease. Aim: The aim of the present study is to explore the effects of submaximal exercise in hypertensive patients focusing on the above mentioned disorders. Patients and Methods: Twenty (20) non-diabetic patients with newly-diagnosed essential hypertension (mean age 55±10 years, 11/9 male/female, mean office blood pressure 157/93 mmHg) participated in one 45 min submaximal exercise test on a bicycle ergometer. Blood samples were taken immediately before and after exercise and one hour after completion of the exercise. The following blood parameters were determined: a. Prothrombin time (PT), activated Partial Thromboplastin time (aPTT), D-dimers, Antithrombin III (ATIII), Protein C (PrC), Prothrombin fragments 1+2 (PF1+2), Thrombin-antithrombin III complex (TAT) and factors VII and XII as indexes of activation of the coagulation cascade b. plasm-inα2 antiplasmin complex (PAP) as fibrinolytic index, c. platelet factor 4 (PF4) and β-thromboglobulin (β-TG) as indexes of platelet aggregation, d. soluble thrombomodulin (TM) and von Willebrand factor (vWf) as indexes of endothelial function e. White blood cell count (WBC), Fibrinogen (Fib) and free antigen of protein S (PrS) as inflammatory markers. Results. All patients completed the exercise test successfully. PAP significantly deviated throughout the whole exercise protocol (pre: 190.1±39 µg/L, exercise: 410.7±225 µg/L, after: 276.5±52 µg/L, p<0.001). A similar deviation was observed in WBC (pre: 6654±1118 µL, exercise: 7285±1589 µL, after: 9552±2310/µL, p<0.001) due to an increase in polymorphonuclear count. We noticed significant differences between pre- and immediately after exercise levels in aPTT (36±5sec vs 33±4 sec, p<0.001), Fib (361±68.4mg/dL vs 396.3±65mg/dL, p<0.001), β-TG (107.3±52 IU/mL vs 148±52 IU/mL, p<0.001), TM (4.9±1.7ng/mL vs 4.2±1.4ng/mL, p=0.009) and vWF (114.6±48% vs 138.9±59%, p=0.008). Levels of ATIII (56±5.5sec vs 38.5±2.4 sec, p=0.001), factor VII (79.2±12% vs 74.7±10%, p=0.001) and vWf (114.5±48% vs 137.7±59%, p=0.007) differed significantly one hour after exercise as compared to pre-exercise levels. PrS was the only parameter that decreased significantly 1 hour after exercise as compared to immediately after exercise levels (98.8±17.7% vs 95±16%, p=0.006). No significant changes were observed in the levels of PT, D-d, ATIII, PrC, TAT, PF1+2, PF4, and factor XII. Conclusions. Submaximal exercise caused an exacerbation of the endothelial dysfunction and the inflammatory state, already existing in never treated hypertensive patients, as part of the atherosclerotic process. However, it seems that the prominent mechanisms were fibrinolytic and inflammatory rather than activated coagulation, since no hypercoagulable state was detected. Additional research, preferably directed towards inflammatory parameters, is required to determine the possible favourable effects of a chronic submaximal exercise programme in hypertensive patients.
review the information on all published cases of argatroban use during cardiac surgery in adults. Aim. The aim of this study is to develop guidelines for the appropriate use of argatroban during adult cardiac surgery. Methods. The information on all reported adult cases of argatroban use during cardiac surgery was reviewed. This analysis focused on patient characteristics, type of surgery, argatroban dosing schedule, monitoring of anticoagulation, morbidity and mortality. Results. Twenty-one cases have been reported. Fourteen patients underwent off-pump surgery. The dose was 5 mcg/kg/min. Intra-operative thrombotic complications were not reported in this group, however, one clot in the pump was noted after the procedure when the ACT was between 300–350 s. All six cases required larger volumes of peri-operative blood products and 3 had severe coagulopathy. Of the 21 cases, 7 had an indication for continued anticoagulation following surgery. Four cases did not report further use of argatroban after surgery. Three patients received argatroban after surgery without complications. Conclusions. Argatroban, with ACT monitoring, can be safely used for anticoagulation during cardiac surgery using the following proposed guidelines. We recommend an ACT level of greater than 300 s for off-pump cardiac surgery and greater than 400 s for CPB. It would be advisable to use an arbitrary upper limit of ACT for both on and off-pump procedures to prevent severe coagulopathy. ACT monitoring seems to be a clinically reliable test to predict coagulation status in patients undergoing cardiac surgery with argatroban and should be checked often (i.e. every 15 minutes) to allow for proper adjustments of the dose. Prospective studies to evaluate the optimal dose and monitoring effect of this agent during cardiac surgery should be supported.

References

PILOT STUDY TO ESTABLISH PREFERENCES TOWARDS COAGULATION FACTOR CONCENTRATES USED TO TREAT HAEMOPHILIC PATIENTS WITH INHIBITORS

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Background. Haemophilia is a very expensive disease. This situation becomes extreme when patients develop inhibitors that compromise the effectiveness of treatment, with potential increase of morbidity and mortality. Treatment of haemophilia is the result of interactions between patients, physicians, pharmacists and budget holders, each carrying their own set of preferences. Aims. A pilot study was conducted to identify which characteristics of coagulation products are considered more important to treat patients with inhibitors: these characteristics will be included with a price proxy characteristic in a Discrete Choice Experiment, with the objective to elicit preferences and willingness to pay towards the treatment modality, in order not to lose information, where one characteristic is considered standing these differences as important is to guide optimal therapeutic strategies in patients with inhibitors.

Table 1. Proposed guidelines for argatroban use during cardiac surgery in adults.

<table>
<thead>
<tr>
<th>Off pump cardiac surgery</th>
<th>On pump cardiac surgery</th>
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<tbody>
<tr>
<td>Argatroban dose</td>
<td>5 mcg/kg/min infusion</td>
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<tr>
<td></td>
<td>Adjust according to ACT</td>
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<tr>
<td>Target ACT</td>
<td>&gt;300s</td>
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<tr>
<td>Monitoring</td>
<td>ACT every 15 min</td>
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<td>Low ACT level</td>
<td>300 s</td>
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<td>High ACT level</td>
<td>500 s (arbitrary level)</td>
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<td>Risks</td>
<td>Intra-operative thrombi</td>
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PRELIMINARY RESULTS FROM THE EUROPEAN ESCHOOL FIELD STUDY

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Background. The ESCHOOL Study is a retrospective/prospective prevalence based cohort study of clinical, quality of life and health economic outcomes in haemophilia treatment in Europe and is sponsored by the EU. 1732 patients with haemophilia from 4 years on have been recruited from 19 European countries, out of them 78% have been enrolled in the study. The ESCHOOL Study consisted of a pilot testing phase where the study procedure was feasibility tested in 70 patients and a field testing phase in which more then 1,300 haemophilia patients participated. Aims. Objectives of the study were a) to make validated instruments available for the assessment of patients’ health status, quality of life and health care and its cost on an European basis, b) to identify models of health care of haemophiliacs in terms of clinical characteristics, their possible costs and impact on quality of life, c) to provide policy recommendations for optimal care of haemophilia patients based on clinical, quality of life and health economic information. Methods. In the ESCHOOL Study patients with haemophilia were asked to complete a comprehensive questionnaire concerning socio-demographic (such as age, gender), psycho-social (such as quality of life, coping) and health economic (such as days lost of work, costs of care) information and to fill in a diary during a 6-months period. In parallel, clinical information concerning bleeding history, inhibitor history, concomitant disease, arthropathy assessment, surgery, treatment modality, type of product and medical visits was obtained from physicians. Results. In the pilot testing 70 patients (41 adults, 29 children) were included from the steering committee countries (Italy, Germany, Romania, Hungary, U.K.) and France. According to the feedback evaluation, patients and parents found the questionnaire good and interesting, but many said it was too long and repetitive. All respondents found the questions relevant for haemophilia. The extended completion time (55 min) led to the decision to shorten the questionnaire for parents and adults. The pilot testing questionnaire was divided into two parts to lose information, where one part was administered at baseline the other part was given at follow-up. Suggestions from physicians were implemented in the medical documentation. In the field testing 1,345 patients (931 kids, 1,412 adults) were enrolled from 19 countries. 87% had haemophilia A and were severely affected (72%). In 11% inhibitors occurred and one third of the patients received prophylactic treatment. 45% of the patients suffered from chronic pain and 40% reported target joints. Viral infections were found in 42% of the patients (hepatitis C) and 8% for HIV. 5% of the patients underwent an orthopaedic surgery. Conclusions. The feasibility testing of
the study documents revealed that the original questionnaires had to be modified for the field testing. Preliminary results of the field testing revealed differences between countries concerning clinical status and treatment modalities and their impact on costs and quality of life. These results underline the importance of the aim of the ESCHoQol. Study, to compare QoL outcome of haemophilia care in Europe in order to recommend future improvements.

0416
INTRACRANIAL HEMORRHAGE IN HEMOPHILICS RECEIVING NO PROPHYLACTIC REPLACEMENT THERAPY
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¹University of Medicine and Pharmacy, TIMISOARA, Romania; ²Ludwig Maximillians University, MUNCHEN, Germany

Background. Intracranial hemorrhage (ICH) in hemophiliacs is the primary cause of death through bleeding. The mortality risk associated with ICH was 70% before the introduction of cryoprecipitate; it decreased to 25-30% thereafter but remained still very high. Aims. Analysis of the frequency, type, severity, and consequences of the ICH in hemophiliacs from Romania, receiving no prophylactic replacement therapy. Methods. The study was conducted on a cohort of 212 hemophiliacs, 180 with hemophilia A, and 32 with hemophilia B, 51.89% of whom was well documented.

To prevent ICH, the efficiency of early home therapy in case of head trauma was determined. The efficiency of prophylactic treatment in case of birth trauma or head trauma. Although the risk for permanent damages of the frequency, type, severity, and consequences of the ICH in hemophiliacs is the primary cause of death through bleeding. The mortality risk associated with ICH was 70% before the introduction of cryoprecipitate; it decreased to 25-30% thereafter but remained still very high. Aims. Analysis of the frequency, type, severity, and consequences of the ICH in hemophiliacs from Romania, receiving no prophylactic replacement therapy. Methods. The study was conducted on a cohort of 212 hemophiliacs, 180 with hemophilia A, and 32 with hemophilia B, 51.89% of whom was well documented.

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men and 11 women; median age 67 years, range 40-85), distributed according to PAI-1 genotype (5G/5G versus 4G/5G or 4G/4G). Not to interfere with the PAI-1 plasma concentration, patients receiving tranexamic acid during the intervention were excluded. Among others, main recorded data included total hemorrhage volume, corporeal temperature, total dose of heparin and protamin, prothrombin time (PT) and activated partial thromboplastin time (aPTT), fibrinogen, d-dimer, antithrombin. Fibrin, partial thromboplastin and fibrinogen concentrations. Four moments were chosen to analyse the different parameters: basal, arrival to intensive care unit, after 4 hours of arrival and after 24 hours. Laboratorial and statistic methodologies are explained. Results. Patients carrying the PAI-1 4G allele (both homozygous and heterozygous) presented a smaller bleeding risk with respect to the homozygous 5G/5G, with significant differences in bleeding at the first 4 hours (532±223 vs. 846±519 mL; p=0.002) and 24 hours after arriving at the intensive care unit (771±446 vs. 1379±582 mL; p=0.016). This smaller hemorrhagic risk correlates with significantly elevated PAI-1 levels at the moment of arrival at the unit in the patients carrying 4G allele (120,3±103 vs. 56,9±7,7 ng/mL; p=0.019), and also higher levels of antithrombin (p=0.016), PT (p=0.019) and fibrinogen (p=0.027), and a higher corporal temperature (p=0.011). We did not find significant differences between both groups of patients for the rest of analysed parameters; all the results are exposed. Conclusions. Some authors have studied the relationship between FXI polymorphism and the risk of ischemic and the risk of ischemic (mainly coronary disease and ictus), but few reports exist that clinically correlate this polymorphism with hemorrhage. We work demonstrates that patients undergoing cardiac surgery who are carriers of the PAI-1 4G allele, may have a significantly lower bleeding risk in the first 24 hours after surgery, when they are compared with homozygous 5G/5G. Although preliminary, these findings are of interest to deduce that patients carrying the 4G allele could not need antifibrinolytics, and thus, to contribute to avoid possible thrombotic complications.

0420
FACTOR XI DEFICIENCY AND POST-PARTUM HAEMORRHAGE: BLEEDERS AND NON-BLEEDERS!
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Management of pregnant women with Factor XI deficiency poses a challenge to the clinician because of the variable bleeding tendency and risks associated with factor replacement. Factor XI deficiency is an uncommon bleeding disorder with an autosomal inheritance. The gene frequency is unknown, comprising only 7% of bleeding disorders in our local data base in the East Midlands (UK). In heterozygotes, there is mild or moderate reduction in Factor XI level, between 20-70 u/dL. Homozygotes or compound heterozygotes have severe reduction in levels, often <1 u/dL. There is no clear correlation between FXI level and bleeding tendency. It is generally trauma or surgery-related. There are few data on pregnancy complications and FXI deficiency. Our study objectives were to assess pregnancy outcome with respect to miscarriage rate and post-abortal bleeding, and post-partum haemorrhage. Since symptoms of bleeding disorders can be difficult to assess objectively, we applied the criteria employed by Bolton-Maggs. Two haematologists independently assessed each set of case-notes and classified each patient into either bleeder or non-bleeder groups. 34 women were identified on the local data-base. Thirty-one had moderate or mild deficiency, while 3 had severe reduction in FXI levels. The patients were evenly divided between bleeders (B) and non-bleeders (nB), with 18 and 16 respectively. In 9 cases, the women had not undergone surgical or dental challenges, and were classified as non-bleeders in the absence of menorrhagia /or mucous membrane bleeding. They had a total of 109 pregnancies, with 79 live births. Pregnancy and delivery was uneventful in the majority of cases, 71% overall (76% nonB; 65% B). Of those pregnancies resulting in a live birth 90% were uneventful (92% nonB; 72% B). The local incidence of PPH is 5%. The total number of instances of PPH in our study was ten (13%), 9 primary and 1 secondary. This increased incidence of PPH was statistically significant, with a p value of 0.029. All but two episodes occurred in the group of women with increased bleeding tendency. Of the women in this group, PPH occurred twice in 2 patients and once in 4 women. When the incidence in bleeders was compared to that of non-bleeders there was a highly significant difference (p 0.000001).In this study, 10 women suffered a total of 13 spontaneous miscarriages. One further woman had a total of 15 miscarriages and was excluded from this analysis. The total rate of miscarriage between 8 and 13 weeks, locally, is 10%. Eleven of the miscarriages in our study were within this time period. The twelfth miscarriage occurred at 22 weeks, in a young woman who had ruptured her membranes at 20 weeks. The cases appeared evenly divided between the two groups. However, significant post-abortal bleeding was noted in 2 cases, both bleeders. In summary, although uneventful for the majority of women, factor XI deficiency caused pregnancy complications for one bleeding subgroup, with a bleeding rate of 13.5%. Further studies are required to define the underlying factors of this group.

0421
USE OF LOW DOSE RECOMBINANT ACTIVATED FACTOR VII INFUSION FOR TREATMENT AND PROPHYLAXIS OF BLEEDING EPISODES IN SEVERE FACTOR VII DEFICIENCY
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Introduction. Severe factor VII deficiency is a rare bleeding disorder which can be treated with fresh frozen plasma, prothrombin complex concentrates and plasma derived factor VII. Recombiant activated factor VII (rFVIIa, Novoseven) is now licensed for the treatment of factor VII deficiency, at a recommended dose of 15-30 micrograms per kilogram (mcg/kg) by bolus injection every 4-6 hours. Correlation is poor between factor VII activity and haemostasis, but levels of 10-15% of normal are generally sufficient to achieve haemostasis. Activated factor VII makes up 1% of the total and in combination with tissue factor is the initiator of coagulation. The licensed dose of rFVIIa raises plasma levels of factor VII far above the physiological norm. The half-life in plasma is short (2-30-2-97 hours) requiring frequent bolus injections. Infusions of low dose rFVIIa seem attractive as it abolishes peak and trough levels and avoids exposure to blood borne viruses and prions. Patients and methods and outcomes. Six patients (5 children and 3 adults) with severe factor VII deficiency (<1-4%) were treated with rFVIIa, totalling 13 episodes (7 bleeding episodes and 6 elective invasive procedures) between July 2003 and January 2006. Five patients are included in Table 1. The sixth patient presented at 5 weeks old with an intracranial haemorrhage.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Patient</th>
<th>Age/sex</th>
<th>Dose Mcg/kg/24 h</th>
<th>Length Days</th>
<th>Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.5 M</td>
<td>300</td>
<td>4</td>
<td>Peri-anal tear ntc transection</td>
<td>Multiple dental extractions</td>
</tr>
<tr>
<td>2</td>
<td>8 y</td>
<td>75</td>
<td>1</td>
<td></td>
<td>Termination of pregnancy</td>
</tr>
<tr>
<td>3</td>
<td>29 F</td>
<td>17.1</td>
<td>3</td>
<td></td>
<td>Normal vaginal delivery</td>
</tr>
<tr>
<td>4</td>
<td>22 F</td>
<td>19</td>
<td>3</td>
<td></td>
<td>Normal vaginal delivery</td>
</tr>
<tr>
<td>5</td>
<td>33 y</td>
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<td>1</td>
<td></td>
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</tr>
</tbody>
</table>

She was treated with a bolus of 60 mcg/kg followed by an infusion of 250 mcg/kg/24 hours for 24 hours. During this time she underwent insertion of an extra-ventricular shunt and of a central venous catheter. There was no excessive bleeding. She has required six further infusions (2-7 days duration) for hip bleeds, gastro-intestinal bleeding and central venous catheter changes with no evidence of excessive bleeding, at doses of 150-250mcg/kg per 24 hours. In emergencies the patients were given a bolus of rFVIIa (60 mcg/kg) immediately followed by an infusion of rFVIIa (1.2 mg diluted in 24 mL 0.9% saline) until the bleeding was controlled. The dose 1.2 mg in the infusions is dictated by this being the smallest vial currently available. In elective procedures an infusion of rFVIIa (17.1-300 mcg/kg/24h) was commenced 2-4 hours prior to procedure. Results and Discussion. Our results show there were no episodes of increased blood loss over the expected for the procedure. There were no episodes where extra doses of rFVIIa or other treatment were required. Therefore we conclude it is feasible, safe and effective to use low dose rFVIIa infusions in both emergency and elective situations. Using doses of 20mcg/kg per 24 hours via an infusion pump appears to be as effective as the licensed dose of 60-180 mcg/kg per 24 hours in adults and may reduce the theoretical risk of thrombo-embolic phenomena. The reduction in dose represents a large cost saving and requires less medical and nursing time refilling the infusion every 24 hours. It may be possible to further reduce the dose given in paediatric patients with smaller vial sizes or if the rFVIIa is shown to be stable for a longer period once reconstituted.
EVALUATION OF BONE MINERAL DENSITY IN CHILDREN WITH HEMOPHILIA: MANSOURA UNIVERSITY CHILDREN HOSPITAL (MUCH) EXPERIENCE: MANSOURA, EGYPT

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Mansoura University Children Hospital, MANSOURA, Egypt

Background. Patients with hemophilia may be at risk for developing reduced bone mineral density for a number of reasons such as recurrent hemorrhosis and immobilization. Aim of the Work. To assess the bone mineral density (BMD) in children with hemophilia and, to correlate bone mineral density with findings regarding the joint disease (hemophilic arthropathy). Patients and Methods. Thirty hemophilic patients aged 4.97±3.64 years and 30 control healthy individuals (had no joint disease) aged 5.09±3.64 years were selected from the hematology unit and outpatient clinic of MUCH respectively. Anthropometric measurements were done to all cases. Z-score was used for weight, height, and Body Mass Index (BMI). Joint evaluation for hemophilic patients and controls was done using Colorado PE-0.5: Half Point Instrument before using Dual Energy X-ray Absorptiometry (DEXA). DEXA scanning was performed to all hemophilic patients and controls focusing on L2-L4. Results. There was no significant difference between hemophilic patients and controls as regard anthropometric measurements and their z-score. There was a significant difference between severe hemophilic patients (factor level assay less than 1%) and controls as regard BMD and BMD z-score (p=0.05, 0.003) respectively. There was a significant difference between severe hemophilic patients (factor level assay less than 1%) and controls as regard BMD and BMD z-score (p=0.01, 0.001) respectively. Also, in hemophilic patients, there was a relevant correlation between joint evaluation scores and BMD z-score (r=-0.365, p=0.04). Conclusions. Children with hemophilia could have reduced bone mineral density compared with age and gender matched controls. This reduction in bone mineral density was independent on difference in age and body size. Children with more established hemarthropic arthropathy exhibited the lowest BMD and BMD z-score.

EVALUATION OF BONE MINERAL DENSITY IN CHILDREN WITH HEMOPHILIA: MANSOURA UNIVERSITY CHILDREN HOSPITAL (MUCH) EXPERIENCE: MANSOURA, EGYPT


0423 CLINICAL AND LABORATORY PECULIARITIES IN CHILDREN WITH VON WILLEBRAND DISEASE

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Von Willebrand disease (vWD) is the most widespread (frequency 1%) hereditary form of hemorrhagic diathesis after hemophilia. The main causes of bleeding in patients with vWD are quantitative and qualitative defects in von Willebrand factor (vWF). Alteration of vWF of platelets has an influence on severity of disease symptoms. The prominence of the bleeding varies due to complicated pathogenesis and variability of the forms. The aim of this study was to establish the clinical and laboratory peculiarities of vWD in children. Materials and Methods. We have assessed hemostasograms in 150 children with vWD aged from 6 mo to 14 years, including 35 children with parents (13 fathers and 22 mothers). Results and discussion. vWD was more frequent in girls (60%). In majority of patients diagnosis was established during initial examination, in 45% during the second examination. Among patients were 2 infants, 8 children of 1-3 y.o., 11 of 4-6 y.o. and 14 patients aged from 7 to 14 years. An evaluation of patients genealogy has allowed to confirm familial character of the disease in majority of patients 84%, an autosomal-dominant type of inheritance was observed in 60%. The first symptoms of the disease were appear in earlier childhood (44%) and in childhood (52%). The main symptoms of the hemorrhagic syndrome were: nasal bleeding (92%), skin hemorrhages (40%), post-operative and post-traumatic bleeding (36%), subcutaneous hemorrhages (18%), tooth (18%) and gingival (6%) bleedings. Metrorrhagias were leading symptoms in 11 girls aged 12-14 years. Intensity of the bleedings was moderate in the majority (65%) of children. Disease course was characterized with periods of bleeding and remission of various duration. The frequency of bleeding episodes was: monthly in 12%, several times per year in 24%, annually in 36%, more rare in 28%. Laboratory investigations were started from routine test: bleeding time (BT) and number of platelets (P). BT was increased in 68% of patients, P was 150-200 thousands/L in all patients. Diagnosis of vWD was established on results of coagulogical investigations: vWF coagulation factors levels and platelet functions. Platelet adhesion to glass (normal value 30-40%) was decreased: in 12% of patients was 0%, in 20% was 1-10%, in 25% - 10-20% and in 40% of patients was 20-30%. Ristomycin-induced (1.2 mg/ml) platelet aggregation was decreased in all patients: in 72% was up to 17 sec, in 28% of patients up to 20 sec (normal value ≤ 12 sec). Activated partial thromboplastin time was changed: in 45% of patients to 55-60 sec, in 28% to 61-60 sec, in 16% to 71-80 sec and more than 80 sec in 8% of patients. 50% decreasing of factor VIII was noted in 50% of patients: in 27% to 20-50%, in 23% of patients to 11-20%. The most accurate indicator in diagnosis of vWD is estimation of blood vWF level. In the majority of children decreasing of this indicator was observed in 10-20% in 15% of patients, 20-40% in 12%, 40-60% in 36% and 40-60% in 40%. So, in the majority of cases vWD type I (plasma vWF protein decreasing) and type III (complete absence of vWF protein) were noted. Almost normal vWF level (60-80%) does not exclude vWD type II. In this case diagnosis can be established by vWF multimeric structure investigations, which not available in our laboratory. Same clinical and laboratory changes were observed in both children and parents in 28 cases, in 7 cases more prominent changes were noted in parents in comparison with children. Thus, von Willebrand disease is one of the most widespread hemorrhagic diatheses with autosomal-dominant or, more rare, autosomal-recessive inheritance. Variability of the clinical and laboratory signs is typical for vWD. vWD can be suspected in cases of familial bleedings in both genders. Relapsing spontaneous nasal bleedings-more frequent symptom of the disease. In patients with vWD vascular-platelet hemostasis alterations are seen: bleeding time increasing, ristomycin-induced platelet aggregation decreasing. Alterations of coagulative chain of hemostasis are characterized by factor VIII activity decreasing and moderate increasing of activated partial thromboplastin time. Most accurate investigation in diagnosis of von Willebrand disease in von Willebrand factor level estimation.

0424 PLATELET FUNCTION TESTING IN URÆMIC PATIENTS

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Background. Chronic renal failure (CRF) is associated with excessive bleeding. Platelet dysfunction is probably the most consistent and important feature, particularly platelet-platelet and platelet-vessel wall interactions. The skin bleeding time (SBT) is the best-established predictor of haemostasis, but have not been specifically assessed in uraemic patients. Aim of the study. A pilot study to examine various in vitro assays to assess platelet function in patients with chronic renal failure. Methods. We include platelet function analyzer (PFA-100), cone platelet analyzer (CPA), whole blood platelet aggregation (WBPA) studies and thromboelastography (TEG). These have been compared to the traditional in vivo assay of skin bleeding time. Methods. Single centre, prospective cohort study of patients referred to a tertiary nephrology unit. Patients with both acute and chronic renal impairment were recruited. Laboratory parameters analysed included full blood count, serum creatinine and urea, calculated GFR (Cockcroft formulation), APTT, PT, fibrinogen, SBT (Simplate II), WBPA, PFA-100, TEG and CPA. If patients were on haemodialysis, blood samples were obtained via tunneled vascaths with heparin removed, or via arterio-venous fistulae pre-dialysis. Results. This study included 42 patients: 9 with CRF (GFR<30 mL/min) not receiving dialysis; 23 CRF patients 30 patients had abnormal TEG tracings, but these were poor reproducibility and accuracy. Additionally the common SBT kit (Simplate II) has been withdrawn from sale in Australia. Several newer rapid assays of platelet function are able to provide a means of assessing primary haemostasis, but have not been specifically assessed in uraemic patients. Conclusions. Various in vitro assays to assess platelet function were compared to the traditional in vivo assay of skin bleeding time.
prothrombotic findings (high MA and increased G parameter), 6 patients underwent renal biopsies with one bleeding complication. This patient had a normal SBT and CPA at the time. Conclusions. In this pilot study we found that prolonged SBT were not predicted by serum creatinine or calculated GFR. Within the limitations of this study, an alternative in vitro test to replicate the SBT has not been identified.

Table 1. Comparison of assays.

<table>
<thead>
<tr>
<th>SBT</th>
<th>PFA-100</th>
<th>WBPA</th>
<th>CPA</th>
<th>TEG</th>
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<tbody>
<tr>
<td>&lt;7min</td>
<td>12</td>
<td>3</td>
<td>11</td>
<td>3</td>
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<td>&gt;7min</td>
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<tr>
<td>&gt;10min</td>
<td>10</td>
<td>2</td>
<td>11</td>
<td>9</td>
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</tbody>
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**0425**

ANALYSIS OF CLINICAL AND BIOLOGICAL FACTORS ASSOCIATED TO EXCESSIVE BLEEDING IN CARDIAC SURGERY


Hospital Universitario de Canarias, LA LAGUNA - TENERIFE, Spain

Background. Bleeding is the most frequent and important complication associated to cardiopulmonary bypass (CPB) in cardiac surgery. The knowledge of physiopathological aspects related to the own CPB and the use of certain measures (less deep hypothermia, drugs like aprotinin, and better control of the intraoperative anticoagulation, among others) have significantly reduced this hemorrhagic risk. Excessive postsurgery bleeding (EPSB) takes place when the hemorrhage volume is superior to 1 liter in the first 24 hours after surgery. Aims. We have analysed what clinical and biological factors are associated to EPSB after cardiac surgery with CPB. Methods. We studied 26 patients undergoing cardiac surgery with CPB (15 men and 11 women; median age 67 years, range 40-85), in whom tranexamic acid was not administered during the intervention. Twelve coronary artery bypass operations, 10 valve repairs, and 4 mixed surgeries, were included. Those patients with EPSB were grouped as opposed to those who did not have EPSB, and differences between both groups in relation to physical factors (corporal temperature, hemostatic parameters and leptin included) and hemogram findings, hemostatic parameters, transfusional requirements and used drugs, were analysed. Data were recorded at four moments: preoperative, arrival at the intensive care unit, after 4 hours of arrival and after 24 hours. The different used statistical tests are explained. Results. EPSB was observed in 13 patients (50%). In the preoperative moment, there were no differences between both groups, except for a lower plasma concentration of PAI-I in the group of patients who showed EPSB. In the moment of arrival at the intensive care unit, those patients who made EPSB presented lower levels of C1q, C1 inhibitor, C7, Factor B of the complement, PAI-I, PT, and leptin, and a lower corporal temperature. After 4 hours of arrival, the patients with EPSB presented lower levels of C1q, C1 inhibitor, C3, C7, Factor B, leptin, PT and fibrinogen. Finally, after 24 hours of arrival at the intensive care unit, the values of C1q, C4 and leptin, were significantly lower in the EPSB-group. We did not find differences in the following factors and parameters: lactate, lysine, interleukin-6, soluble TNF receptor, APTT, antithrombin, PT, BUN, creatinine, leukocytes, platelets, PK-NAC and CK-MB, administered dose of dobutamine and noradrenaline, and haemodynamic indexes (cardiac index and systemic vascular resistance index). Conclusions. In our experience, several biochemical and hemostatic parameters could serve as predicting factors of EPSB in patients undergoing cardiac surgery. Specifically, some factors of the complement system and leptin (obesity-related protein) seem to play an important role. Our work supports that the activation of the complement system caused by the CPB, could play an important role in the postsurgery hemorrhage.

**0426**

SUBJECTIVE TRAINING EFFECTS ON ADULT PATIENTS WITH HAEMOPHILIA ATTENDING A SPORTS THERAPY PROGRAMME

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Background. Only since some years sport activities have been recommended for haemophilia patients. Still now the importance of sports therapy as an integral element in haemophilia treatment has not yet been widely recognized. In the frame of the Haemophilia & Exercise Project (HEP) the success of a two years sports specific therapy was evaluated subjectively in terms of isometric muscular strength and proprioception and subjectively in terms of the WOMAC questionnaire and the orthopaedic joint score. Subjectively perceived training effects were tested with the a newly developed sport-specific questionnaire (HEP-Test). In addition quality of life was tested with the SF-36 and the haemophilia-specific quality of life questionnaire (Haem-A-Qol). Aims. Assessment of subjective training effects of a sports therapy programme for adult patients with haemophilia in terms of bodily condition and quality of life. Methods. Based on the contents of the training programme a sport-specific questionnaire (HEP-Test) was developed consisting of 33 items pertaining to 6 dimensions (physical status, mobility, strength & coordination, endurance, body perception, general questions). HEP-Test was pilot tested in 23 German adult haemophilia patients and tested for it’s feasibility in terms of acceptance, comprehensibility and relevance. Data were psychometrically analysed in terms of reliability and validity (criterion, convergent, discriminant). Correlation of the HEP-Test with subjective and objective measures were performed. Results. From the 23 enrolled patients 87% were severely affected by haemophilia. In 8.7% inhibitors occurred and half of the patients received prophylactic treatment (52.2%). 47.8% of the patients reported target joints. Viral infections were found in 65.2% of the patients (hepatitis C) and in 21.7% for HIV. Concerning the newly developed HEP-Test the mean completion time was 15 minutes; the questionnaire was well accepted and patients found it related to physical activities. Feasibility testing led to the omission of 9 items and suggestions for wording of some items were given by patients. Psychometric testing revealed excellent characteristics for reliability (Cronbach’s α ranging from .82-.90). Validity testing showed high correlation between scales of HEP-Test, SF-36 and WOMAC. Acceptable to high correlation were found with the orthopaedic joint score and the isometric muscular strength test. Discriminant validity testing revealed significant differences for clinical subgroups. Conclusions. HEP-Test is a short questionnaire assessing subjective training effects. HEP-Test was well-accepted by patients and showed quite satisfactory psychometric characteristics. Subjective training effects can be measured with the HEP-Test and should be combined with objective assessments in order to reveal aspects, which can not be measured objectively such as body perception.

**0427**

THE USE OF FEIBA AND NOVOSEVEN FOR TREATMENT OF BLEEDING EPISODES IN PATIENTS WITH HEMOPHILIA A AND FACTOR VIII INHIBITOR: A SINGLE CENTRE EXPERIENCE

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Background. Factor VIII replacement is impossible in hemophiliacs with high titre inhibitor, so FEIBA® and NovoSeven® are the main possibility in treatment of bleeding. Aims. Evaluate efficacy and consumption of the products in treatment of bleeding episode in hemophiliacs with factor VIII inhibitor. Methods. We used data accumulated in our hemophilia centre in the course of 6 years between 2000 and 2005. Five hemophiliacs with factor VIII inhibitor were treated on demand with FEIBA® (40-79 U/kg; 8-12 h) or with NovoSeven® (dosage 200-210 μg/kg à 2 h). For efficacy we used evaluation criteria: excellent: bleeding stops within 8 hours from start of treatment, efficient: bleeding stops more than 8 hours following start of treatment, partially efficient: bleeding stops but recurring within 48 hours following stop of bleeding, inefficient: no stop of bleeding after 48 hours of treatment or need for another treatment. Results. Patients had 124 bleeding episodes, included 99 spontaneous bleeding episodes (88 hemarthroses, 6 muscle bleedings and 5 other sites bleedings) and 17 traumatic bleedings (9 hemarthroses, one muscle bleeding, 2 other sites bleedings and 5 multiple sites
bleeds) and 8 re-bleeding episodes. Bleeding episodes were treated mostly with NovoSeven® (78) and with FEIBA® (45). We evaluated all episodes (except the re-bleeding episodes) treated with NovoSeven® (71) or with FEIBA®(44). Median total dose per episode was 352 µg/kg and 190 U/kg, dose per infusion was 112 µg/kg and 60 U/kg. In episodes with re-bleeding treated with NovoSeven® (6) median total dose per episode was 382 µg/kg, dose per infusion was 110 µg/kg. Using FEIBA® one episode with re-bleeding had occurred total dose 95 U/kg, 47 U/kg per infusion (dosage was lower than average). The efficacy of NovoSeven® and FEIBA® was excellent in 70% and 47.7% of the episodes, efficient in 21.4% and 47.7%, partially efficient in 8.6% and 2.3%, inefficient in 0 and 2.3%, respectively. We evaluated spontaneous hemarthroses. NovoSeven® was used in 54 episodes and FEIBA® in 54 episodes. Median total dose per episode was 352 µg/kg and 187 U/kg, dose per infusion was 112 µg/kg and 60 µg/kg. In episodes with re-bleeding treated with NovoSeven® (5) median total dose per episode was 386 µg/kg, dose per infusion was 112 µg/kg. Using FEIBA® only one episodes was with re-bleeding it had been mentioned above. The efficacy of NovoSeven® and FEIBA® was excellent in 70.4% and 47.1% of the episodes, efficient in 20.4% and 50%, partially efficient in 9.2% and 2.9%. Median (mean) interval between start of bleeding and start of treatment in spontaneous hemarthroses treated with NovoSeven® without re-bleeding was 2.4 h (5.9 h), with re-bleeding was 2.5 (2.7 h). Conclusion. In our experience treatment with NovoSeven® stopped bleeding earlier than with FEIBA®, however about 9% of the episodes were with re-bleeding although the dosage used in these episodes and intervals to start of treatment were on the same level as in episodes without re-bleeding. The question is why any bleedings treated in the same way were recurring and the other not.

Orthopaedic status of persons with haemophilia in a developing country

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Background. The medical approach of haemophilia, prototype of rare diseases, can be considered as the reflection of diagnostic and therapeutic performances in this disease. Aims. We sought to perform a cross-sectional analysis of joint status in persons with haemophilia (PWH) is accepted as reliable clinical reflection of diagnostic and therapeutic performances in this disease. Methods. The study was conducted on 93 patients (77 with haemophilia A and 16 with haemophilia B) consecutively enrolled: 31.18% children and young adults; 92.47% with a rest-activity ≤1%. There were significant difference in serum TAFI Ag levels between control group (88.9±16.9%) and in patients with DIC (82.3±14.3%) and in patients with DIC (82.3±14.3%) (p=0.007). Conclusions. We could not show a correlation between the platelet count and VEGF levels. High value of VEGF in serum of the patients may be overt after the disease progression and serial analysis might be helpful. On the other hand TAFI Ag levels were significantly low in patients with DIC. As it has been suggested that TAFI Ag is mainly under genetic control, further combined approach measuring TAFI levels and TAFI gene polymorphism will be needed.

0428
0429

SEVERE VASCULAR ENDOTHELIAL GROWTH FACTOR AND THROMBIN-ACTIVATABLE FIBRINOLYSIS INHIBITOR ANTIGEN IN CHILDREN WITH DISSEMINATED INTRAVASCULAR COAGULOPATHY

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Background. Disseminated intravascular coagulopathy (DIC) is a syndrome characterized by systemic intravascular activation of coagulation resulting in depletion of platelets and coagulation factors. Hypercoagulability and hyperfibrinolysis were both thought to be important mechanisms of DIC. Vascular endothelial growth factor (VEGF) is one of the potent angiogenic polypeptides produced by multiple tissues. High amount of VEGF is stored within circulating platelets and is subsequently released during platelet aggregation. When chronic stimulation of VEGF continues vascular hyperpermeability and thrombosis may be induced. We speculated that extremely high value of VEGF in serum of the patients with DIC might be caused via VEGF release in activated platelets. Thrombin-activatable fibrinolysis inhibitor (TAFI) is considered as a modulator of fibrinolysis and therefore it might play an important role in the pathogenesis of DIC. We planned to evaluate the predictive value of serum VEGF and TAFI for the determination of DIC. Aim. To evaluate clinical and laboratory findings of 40 consecutive children in a single center, diagnosed as DIC according to ISTH criteria and compare serum VEGF, TAFI levels of these patients with 40 healthy objects to clarify their roles in the pathogenesis of DIC. Methods. Forty patients who experienced DIC in our department (Pediatric Hematology Unit of Hacettepe University, Ankara) between December 2005- May 2005 were examined. At the time of diagnosis hemostatic datas of patients with DIC were noted, serum sample of patients with DIC was collected and stored at -80 0 C. Results. The underlying diseases of the patients were congenital heart disease (7 patients), chronic renal failure (5 patients), malignancy (5 patients), metabolic disease (5 patients) and collagen tissue disease (2 patients) Twenty four patients had infection, which 17 of them were documented. Mean acute quantitative CRP level was 6, 0±6, 2. At the time of diagnosis median WBC count was 7050/mm 3 (600-154000), platelet count was 70.000/mm 3 (8000-624000) and hemoglobin level was 9,5 g/dl (5,8-16,3). Low level of protein C and S levels were detected in 18 (32.5%) and 9 (22.5%) patients respectively. Fibrinogen levels were decreased only in six of patients. Majority of patients (57 (92.5%)) had prolonged prothrombin time over 6 seconds. D-dimer levels over 2 g/dl. in were detected in 36 (90%) patients. No significant difference were observed in the VEGF levels between study group (88.9±16.9%) and in patients with DIC (82.3±14.3%) and in patients with DIC (82.3±14.3%) (p=0.007). Conclusions. We could not show a correlation between the platelet count and VEGF levels. High value of VEGF in serum of the patients may be overt after the disease progression and serial analysis might be helpful. On the other hand TAFI Ag levels were significantly low in patients with DIC. As it has been suggested that TAFI Ag is mainly under genetic control, further combined approach measuring TAFI levels and TAFI gene polymorphism will be needed.

This study was supported by Turkish Society of Hematology.
**Dendritic cells and cellular immunotherapy**

**0430** EXPRESSION AND REGULATION OF ENDOTHELIAL PROTEIN C RECEPTOR IN MONOCYTE-DERIVED DENDRITIC CELLS

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**Background.** Endothelial protein C receptor (EPCR) is a transmembrane protein, homologous to MHC class-I molecules, that enhances the rate of protein C activation on endothelial cells. It is reported that EPCR mediates the anti-apoptotic activity of activated protein C on endothelial cells. EPCR was identified also in polymorphonuclear leukocytes and in monocytes. We previously showed by immunohistochemistry that dendritic-like cells in the normal gut mucosa express EPCR. Aims of the study. 1. To characterize phenotypically the gut mucosa EPCR+ dendritic-like cell. 2. To study, in a model of dendritic cell generated in vitro, the expression of EPCR and its modulation. Methods. EPCR was identified by immunohistochemistry, immunofluorescence or flow cytometry. Dendritic cells in vitro were obtained from CD14+ peripheral blood leukocytes, cultured in the presence of interleukin-4 and GM-CSF (MoDCs). Specific messenger RNA (mRNA) was measured by RT-PCR. Results. We confirm that the gut mucosa dendritic-like cells have a phenotype characteristic of dendritic cells, namely they express CD80, CD83 and HLA-DR. We could not identify by immunohistochemistry EPCR+ dendritic cells in other tissues, such as lymph node, spleen, tonsil, liver, lung, and skin. EPCR surface expression on MoDCs was monitored by flow cytometry together with expression of the DC markers HLA-DR, CD1a, CD80 and CD83. After 7 days of culture, approximately 25% of immature DCs expressed EPCR on their surface. De novo expression of EPCR was not correlated with modulation of apoptosis in cell cycle. Lipopolysaccharide-induced terminal maturation of MoDCs down regulated the surface expression of EPCR by 40% while up regulating the expression of CD83. Incubation of cultured DCs with prostaglandin E2 up regulated EPCR mRNA and protein expression by about 3 fold at 20 hours. Flow cytometry studies were compounded by confocal microscopy, which showed that dendritic cell EPCR has the same membrane distribution pattern as in endothelial cells. Conclusions. Contact with bacterial antigens modulates EPCR expression on MoDCs, suggesting EPCR might be involved in antigen recognition or processing.

**0431** HIGH AFFINITY CYTOTOXIC T CELLS CANNOT OVERCOME THE INTRINSIC RESISTANCE TO THERAPY OF (LEUKEMIC) PRECURSOR CELLS IN DORMANCY

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Most patients with leukemia treated with chemotherapy show a good initial response to therapy. However, despite multiple courses of treatment even in patients initially developing a complete clinical response even in patients initially developing a complete clinical response, the disease recurs. The rate of protein C activation on endothelial cells. It is reported that EPCR mediates the anti-apoptotic activity of activated protein C on endothelial cells. EPCR was identified also in polymorphonuclear leukocytes and in monocytes. We previously showed by immunohistochemistry that dendritic-like cells in the normal gut mucosa express EPCR. Aims of the study. 1. To characterize phenotypically the gut mucosa EPCR+ dendritic-like cell. 2. To study, in a model of dendritic cell generated in vitro, the expression of EPCR and its modulation. Methods. EPCR was identified by immunohistochemistry, immunofluorescence or flow cytometry. Dendritic cells in vitro were obtained from CD14+ peripheral blood leukocytes, cultured in the presence of interleukin-4 and GM-CSF (MoDCs). Specific messenger RNA (mRNA) was measured by RT-PCR. Results. We confirm that the gut mucosa dendritic-like cells have a phenotype characteristic of dendritic cells, namely they express CD80, CD83 and HLA-DR. We could not identify by immunohistochemistry EPCR+ dendritic cells in other tissues, such as lymph node, spleen, tonsil, liver, lung, and skin. EPCR surface expression on MoDCs was monitored by flow cytometry together with expression of the DC markers HLA-DR, CD1a, CD80 and CD83. After 7 days of culture, approximately 25% of immature DCs expressed EPCR on their surface. De novo expression of EPCR was not correlated with modulation of apoptosis in cell cycle. Lipopolysaccharide-induced terminal maturation of MoDCs down regulated the surface expression of EPCR by 40% while up regulating the expression of CD83. Incubation of cultured DCs with prostaglandin E2 up regulated EPCR mRNA and protein expression by about 3 fold at 20 hours. Flow cytometry studies were compounded by confocal microscopy, which showed that dendritic cell EPCR has the same membrane distribution pattern as in endothelial cells. Conclusions. Contact with bacterial antigens modulates EPCR expression on MoDCs, suggesting EPCR might be involved in antigen recognition or processing.

**0432** DIFFERENTIATION TOWARDS LEUKEMIC DENDRITIC CELLS IS HINDERED BY THE PRESENCE OF A FLT-3 INTERNAL TANDEM DUPLICATION IN AML BLASTS

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In the search for new treatment modalities to eradicate minimal residual disease cells (MRD) in acute myeloid leukemia (AML), immunotherapy provides an attractive option. AML blasts show differentiation towards leukemic dendritic cells (DC), providing the unique opportunity to generate DC harbouring the full range of tumour antigens. In a large cohort of AML samples (n=154) AML-DC were generated by two culture methods, i.e. in presence of cytokines GM-CSF, TNF-α, SCF, Flt-3L, IL-3 and IL-4 (n=147) or calcium ionophore (CI) and IL-4 (n=108). Median AML-DC yield, defined by phenotypical DC characteristics, in the cytokine-based cultures was 12% (range:0-70%). Considering cultures yielding >10% AML-DC, successful, successful (85/147) of cytokine-based cultures were successful and 61% (66/108) of CI cultures. Overall, functional AML-DC generated with either method was possible in 66% (101/154) of patients. Identification of AML blast populations with DC differentiation capacity is important to select patients eligible for immunisation programs. Interestingly, presence of Flt-3 internal tandem duplication (ITD) was strongly correlated with decreased DC differentiation capacity in both culture methods (cytokine-based culture: p<0.001; CI-culture: p=0.03) suggesting that constitutive activation of tyrosine kinase receptors inhibits differentiation towards DC. In multiparameter regression analysis, powerful predictors for cytokine-based AML-DC culture outcome were presence of Flt-3 ITD (B:-3.41, p<0.001), CD41 (B:3.82, p<0.001) and TNFα-R1 (B:2.82, p<0.001). This regression model predicts 88% of culture outcomes. ROC curves show high sensitivity (95%) and specificity (76%) with an AUC of 0.99 (p<0.001). In 25% of unsuccessful cytokine-based cultures, the CI-based culture method provides an alternative. This percentage increases to 56% if Flt-3 ITD+ AML samples are left out, emphasizing Flt-3 ITD+ blasts’ inability to differentiate towards leukemic DC. In conclusion, AML-DC cultures are successful in most patients. Selection of patients is well possible based upon the presence of Flt-3 ITD and the expression of CD14 and TNFα-R1. Based on these results, we are currently entering patients in a phase I/II clinical vaccination trial.

**0433** THE NOTCH LIGAND DELTA-LIKE1 PROMOTES THE GENERATION OF INTERSTITIAL AND MUCOSAL DENDRITIC CELLS FROM HUMAN MYELOID COMMITTED PROGENITOR CELLS

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The Notch ligand Delta-like-1 (DL-1) and the ETS-transcription factor PU.1 are key regulators of dendritic cell development. We recently observed that ectopic expression of PU.1 in CD34+ cord blood progenitor cells promotes the development of human conventional CD1a+ DCs (cDC), compromising Langerhans cells and interstitial type DCs.

cell fraction, unmanipulated CD34 progenitor cells, and resting T and B cells (not depleted to be protected from CTL-induced cell death (0-20% lysis). To investigate whether these target cells in dormancy were intrinsically resistant to the effector mechanism used by the T cells or that decreased avidity of the interaction between dormant targets and high affinity effectors was underlying the poor susceptibility, we artifically enhanced the avidity by exogenous loading of the target cells with saturating concentrations of the relevant peptide. This was sufficient to completely restore the sensitivity to levels comparable to activated proliferating target cells, suggesting that reduced avidity of the interaction is playing a significant role in the differential killing of activated and dormant target cells. The differential susceptibility of dormant and activated target cells for T cell recognition may also explain the differential cytokine production between T cell killing in the absence or presence of the cytokine storm after alloSCT. We mimicked the cytokine production during GvHD by the addition of interferons. This resulted in a limited, but significant upregulation of the sensitivity of the initially resistant target cell types to recognition by the T cells. In conclusion, we here demonstrate that normal hematopoietic and leukemic cells in dormancy are relatively resistant to cell death induced by high affinity CTL clones. This selective resistance of cells in dormancy is caused by the diminished avidity of the interaction with the CTL.
Vaccination with WT1 and Pr3-derived peptides in patients with AML/MDS and MUC1-derived peptides in patients with multiple myeloma - preliminary results

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Background. It has been demonstrated that the Wilms Tumor gene (WT1) is highly expressed in various types of leukemia. WT1 expression level reflects the extent of minimal residual disease and significantly increases at relapse. Proteinase 3 is an aberrantly expressed myeloid leukemia protein and T cells with specificity for both, WT1 and Pr3-derived antigens, have been generated in vitro from healthy individuals and cancer patients and lysed myeloid leukemic blasts. MUC1(CD227) is presented on a considerable amount of multiple myeloma cell lines and cancer patients and lysed myeloid leukaemic blasts. MUC1(CD227) presented on a considerable amount of multiple myeloma cell lines and plasma myeloma cells, but only some B-cells. Several lines of investigation have provided conclusive evidence that MUC1-derived HLA-class I/II epitopes do represent universal tumor antigens, which are also expressed by malignant plasma myeloma cells and could thus be attacked by MUC1-specific CTLs.

Methods. HLA-A2.1 positive patients with AML/MDS <30% blasts in the bone marrow biopsy receive 6 injections of WT1 and Pr3-derived peptides, combined with Montanide ISA51 (incomplete Freund’s adjuvants), PADOre, and VaxImmune (CpG7909). Vaccination is given every two weeks. HLA-A2.1 positive patients with multiple myeloma Stage I, stable disease or partial remission after chemotherapy receive 6 injections of two different MUC1-derived peptides, VaxImmune and Montanide with or without PADOre. Safety and feasibility as well as clinical course is reassessed every visit. Induction of immune response is assessed by ELISPOT, C6-release-Assays and FACS-analysis (Tetramer-staining). Results. So far, three patients completed our ongoing AML-vaccination protocol; four patients were vaccinated in the myeloma-study. A total of 10 patients will be treated in each trial. Local inflammatory responses at the injection site, such as redness, swelling and pain were observed in all patients (Grade II). In one case, skin necrosis (Grade II) and superinfection occurs. Four out of seven patients developed a systemic reaction including influenza-like symptoms and fever (Grade III). One patient suffered from an anaphylactic reaction (GRADE III) after vaccination. Clinical response data have so far been analysed for patients vaccinated with Pr3 and WT1-derived peptides: Peripheral platelet counts of a patient suffering from MDS RAEB-T improved while blasts detected in the bone marrow remained stable. Two out of three patients with refractory AML remained progressive even after six vaccinations. Clinical responses of patients treated with MUC1-derived vaccine as well as immunological analyses of all vaccinated patients are pending and will be presented at the meeting. Summary / Conclusion. Vaccination with WT1/Pr3 or MUC1, PADOre, VaxImmune (CpG7909) and Montanide is safe and feasible, even in advanced disease and after multiple previous therapies, including stem cell transplantation. Observed local as well as systemic side effects were predominantly mild to moderate. Overall clinical and immunological response data will be presented.

Cancer-testis antigens are commonly expressed in multiple myeloma and induce systemic immunity following allogeneic stem cell transplantation

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Background. Immunotherapies using cancer-testis (CT) antigens as targets represent a potentially useful treatment in patients with multiple myeloma (MM) who commonly show recurrent disease following chemotherapy. Furthermore, CT antigens might represent targets for graft-versus-leukemia (GvL) effects following allogeneic stem cell transplantation (alloSCT). Methods. We analyzed the expression of 11 CT antigens in bone marrow samples from MM patients (N=107) and healthy donors (N=32). Furthermore, we analyzed 66 MM patients for antibody responses against MAGEA3, SSX2, and NY-ESO-1 in an ELISA assay. Finally, we screened a patient with a humoral response against NY-ESO-1 for T cells against the same antigen in an ELISPOT assay using overlapping peptides. Results. CT antigens were frequently expressed in MM with 56% (MAGEC2), 55% (MAGEA3), 35% (SSX1), 20% (SSX4, SSX5), 16% (SSX2), 15% (BAGE), 7% (NY-ESO-1), and 6% (ADAM2, ILIPI) expressing the given CT antigen (see Figure). Importantly, with the exception of SSX4 none of the CT antigens were expressed in healthy bone marrow. Analyzing our patients for IgG antibodies against MAGEA3, SSX2, and NY-ESO-1, we found strong antibody responses against CT antigens in 9 patients who had received alloSCT. Antibody responses against NY-ESO-1 correlated with NY-ESO-1-specific CD4+ and CD8+ T cell responses against peptide NY-ESO-1 51-62 and CD4+ responses against peptide NY-ESO-1 121-140 in one of these patients. These allogeneic immune responses were not detectable in pre-transplant samples and in the patients’ stem cell donors indicating that CT antigens might indeed represent natural targets for graft-versus-leukemia effects. Conclusions. We show here for the first time that CT antigens induce spontaneous antibody and T cell responses in MM patients who received alloSCT. These immune responses induced by alloSCT could probably be boosted by active CT antigen-specific immunotherapy which might help to achieve long-lasting remissions in patients with MM.

Figure 1. Expression of CT antigens in MM.
ed 17.9±6% of all pDC and showed a CD84+/HLA-DR+/CD123+/CD45+ phenotype; intermediate stage pDC (stage II) represented 21±6% of all pDC and they were CD34+/HLA-DR+/CD123+/CD45+ and; the more mature pDC (52±11%) were CD34+/HLA-DR+/CD123+/CD45++ (stage III), Both HLA class I and class II molecules showed a high expression in stage I pDC, which decreased in the intermediate stage to finally recover and remaining expressed at high levels in stage III pDC. Costimulation molecules, BM pDC were negative for CD1a, CD1b, CD1c, CD209, CD275 and the TLR4 and TLR9 Toll-like receptors, while BM pDC were negative for CD1a, CD1b, CD1c, CD209, CD275 and the TLR4 and TLR9 Toll-like receptors, which were expressed at high levels in stage III. CLA was heterogeneously expressed throughout the maturation. Conclusion. In summary, we show that at least three maturation stages of pDC are identifiable in normal adult BM, on the basis of their different phenotypic characteristics, this representing a frame of reference for a better identification and understanding of pDC precursor malignancies.

**0437 COMBINED TYROSINE KINASE INHIBITION AND IMMUNOTHERAPY AS A STRATEGY TO IMPROVE OUTCOME AFTER REDUCED INTENSITY ALLOGENEIC STEM CELL TRANSPLANTATION IN CHRONIC MYELOID LEUKAEMIA**


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Background. The graft-versus-leukaemia effect of allogeneic stem cell transplantation (DLI) is capable of producing long-term disease-free survival in CML. Despite this the toxicity of the conditioning regimen and risk of graft-versus-host disease (GVHD) make allografting an unattractive therapeutic option in most patients. Recently it has been shown that reduced intensity conditioning (RIC) regimens incorporating alemtuzumab reduce both transplant related mortality and GVHD risk. However such regimens are associated with a high rate of relapse which occurs in most patients within the first year post-transplant. Whilst DLI is a safe and effective salvage therapy after a myeloablative transplant, its use is associated with a significant risk of severe GVHD in patients who relapse early after a RIC transplant. Therefore strategies which permit the effective use of DLI after RIC allografts are required if they are to fulfill their curative potential. Aim. We have studied whether the administration of leukaemia specific therapy in the form of cytokine induced killer (CIK) cells may be an attractive strategy for cell therapy of ALL.

Methods. Patients with available sibling donor underwent allogeneic stem cell transplantation using fludarabine 250mg/m2/day iv (days -7 to -5), busulphan 8 mg/m2 orally (days -5, -4) and alemtuzumab 10 mg/day iv (days -7 to -3). Cyclosporin was used as GVHD prophylaxis. Imatinib was commenced on day +35 and continued until one year post transplant at 400mg daily. Minimal residual disease levels were measured by quantitation of BCR-ABL transcript numbers at three monthly intervals. Escalating dose DLI was administered in patients who relapsed after the discontinuation of Imatinib. Results. CML in 1st chronic phase were monitored for a period between March 2002 and September 2005. The median age was 49 (25-57). All patients engrafted promptly and commenced Imatinib on day +35. All tolerated continued treatment until one year post-transplant apart from 3 in whom it was temporarily discontinued because of gastrointestinal intolerance. The day 100 transplant related mortality was 0%. Only one patient developed acute GVHD (grade 3) in all patients treated achieved molecular remission. After discontinuation of Imatinib DLI was instituted in 11 patients because of disease relapse. 4 patients developed GVHD in association with DLI. Summary. We conclude that the combination of Imatinib and a RIC allograft is remarkably well tolerated in patients with CML and may allow the subsequent delivery of DLI without compromising its ability to produce molecular remission.

**0438 CYTOKINE INDUCED KILLER CELLS TRANSCENDENT WITH ANTI-CD19 CHIMERIC RECEPTORS CONTAINING 4-1BB HAVE POWERFUL ANTI-LEUKEMIC ACTIVITY**

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Background. CIK cells are a population of ex-vivo expanded cells with MHC-unrestricted cytotoxicity against several tumoral targets, except B-lineage Acute Lymphoblastic Leukemia (ALL). We have recently demonstrated that transduction of an anti-CD19-ζ receptor in CIK cells rendered them efficient killers of CIK-resistant ALL cells. Conceivably, the capacity to proliferate after contact with leukemia cells and the prolonged anti-leukemic cytotoxicity after infusion should be important to maximize the likelihood of success of this cell therapy. It was previously shown that incorporation of costimulatory molecules into chimeric receptors markedly enhances target-cell stimulated proliferation and cytotoxicity in T lymphocytes and Natural Killer cells. Aims. to identify costimulatory molecules that increase the cytolytic activity and proliferative capacity of anti-CD19-ζ receptor transduced CIK cells. Methods. CIK cells were transduced with a RD114-pseudotyped retroviral vector carrying different types of receptors: anti-CD19-ζ, anti-CD19-DAP10, anti-CD19-4-1BB-ζ and anti-CD19-CD28-ζ, A truncated form of the receptor was used as control. The cytotoxic activity of transduced CIK cells against ALL cells was measured in co-culture experiments with the OP-1, B(3)1,4 cells and, for 4 hours (short-term cytotoxic assay) or for 6 days on a monolayer cell layer (long-term cytotoxic assay). The recovery of ALL cells was evaluated by flow cytometry. CIK cell proliferation was assessed in cocultures with irradiated OP-1 cells and low-dose IL-2. Results. CIK cells were efficiently transduced with the anti-CD19 retroviral vectors (average expression of GFP and chimeric receptor, 55% for all vectors tested; n = 5 each). After 4 hours of incubation, CIK cells expressing anti-CD19-ζ anti-CD19-DAP10, anti-CD19-CD28-ζ and anti-CD19-4-1BB-ζ receptors were all strongly cytotoxic against OP-1 cells (>60% of lysis at E : T ratio 2:1 for all receptors tested). However, the benefits of adding the costimulatory molecules DAP10 or CD28 to the receptor was evident in long-term assays with low percentages of CIK cells (E:T ratio 0.01:1). In these assays, CIK cells expressing anti-CD19-4-1BB-ζ or anti-CD19-CD28-ζ receptors had more potent cytotoxicity than cells expressing the anti-CD19-ζ receptors: in experiments with 4 donors average cell killing was 92.8% (range, 89.4-97.6%); 93.5% (87.0%-96.8%), and 13.8% (2.9%-23.6%), respectively (p = 0.001). By contrast, addition of DAP10 to the receptor did not improve cytotoxicity: average cell killing 2.3% (2.2%-2.4%). Notably, CIK cells transduced with the anti-CD19-4-1BB-ζ receptor had higher proliferative capacity in cocultures with OP1 and low dose IL-2. The average fold increase after 2 weeks of culture was 2.6 (range, 2.4-3.0) for these cells while expansion of cells transduced with either anti-CD19-ζ or anti-CD19-CD28-ζ was 1.4 (range, 1.3-1.5) and 1.7 (1.2-2.7), respectively (p = 0.01). Conclusions. expression of anti-CD19-4-1BB-ζ chimeric receptors in CIK cells confers powerful and specific cytotoxic activity against ALL cells, and induces their proliferation. We suggest that anti-CD19-4-1BB-ζ expressing CIK cells may be an attractive strategy for cell therapy of ALL.

**0439 INTRACELLULAR EXPRESSION ANGiotensIN-CONVERTING ENZYME (ACe, CD143) BY LEUKEMIC DENTRIC CELLS IN ACUTE MYELOID LEUKAEMIA (AML) PATIENTS**

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Background. Dendritic cells (DC) play a key role in the induction of adaptive immune response because they are efficient in antigen presen-
tation and costimulation of naïve lymphocytes. AML blast cells can be induced to differentiate into leukemic dendritic cells (LDC). LDC are quite similar to dendritic cells derived from monocytes of healthy donors (DC) by the expression profile of integrins and co-stimulatory molecules. The only difference between LDC and DC is the absence of surface ACE expression on LDC in contrast to high level of ACE on DC.

Aim. We studied the absence of surface ACE expression on LDC due to the block of normal ACE transport to the cell surface. To confirm this hypothesis we quantified the level of intracellular and surface ACE in LDC and DC. Methods. Blood samples were collected from 10 AML patients at diagnosis before induction chemotherapy and 5 healthy donors (control group). Mononuclear cells were isolated using gradient centrifugation with Ficoll-Paque and were differentiated into dendritic cells by culturing with 180 ng/mL calcium ionophore for 4 days. DC were stained for surface and intracellular ACE using two mAbs to ACE-1 (clone 1D8 for unfolded ACE (mainly located intracellular) and 9B9 for both native (surface) and unfolded ACE and further analyzed by flow cytometry. Results. LDC derived from AML blasts did not express surface ACE 1.5±0.9% of positive cells - clone 1D8 and 2.4±2% - clone 9B9. However, LDC contained a large amount of intracellular ACE: 67±16% (clone 1D8), 81.3±9% (clone 9B9). In contrast, surface ACE expression was revealed in 46.5±5.4% of donors DC (with mAb 9B9). DC derived from monocytes of healthy donors had lower intracellular ACE 10.3±9% of cells (with mAb 1D8) and 5.48±3.1% (with mAb 9B9). The proportion of intracellular ACE expressing LDC was significantly higher than in DC (p<0.001 and p<0.001 for clones 1D8 and 9B9, respectively). The proportion of surface ACE positive LDC was significantly lower than surface ACE positive DC (p<0.001 for clone 9B9). Conclusions. The data demonstrated the absence of ACE transport to the cell surface of LDC and therefore, provide another evidence of the distorted differentiation capacity of AML blasts.

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Background. Immunotherapy represents a promising treatment strategy for many types of cancer. This approach is hampered by the difficulty in generating sufficient number of cytotoxic cells especially in patients heavily treated. CIK cells are a novel population of immune effector cells CD3+CD56+ with high proliferation rate and potent antitumor activity against a variety of tumor cells targets through a perforin based mechanism mediated by NKG2D. We started a pilot clinical trial in patients with refractory lymphoma and metastatic solid tumors according to GMP guidelines that is currently ongoing. The aim of this study is to assess the role of this reinfused CIK cells in the antitumor effect. Methods. CIK cells were generated from PBMC and incubated in LifeCell culture bags in the presence of IFN-γ followed by IL-1β, OKT3 and IL-2. Expansion was assessed between day 21 and 28 and flow cytometric analysis was performed every week. Patients were monitored before and after treatment. Results. We enrolled 11 patients: 6 advanced lymphomas, 4 metastatic kidney carcinoma (RCC) and 1 hepatocellular carcinoma (HC). The median number of transferred cells per patient was 19×10^6 (6.37×10^6) and the absolute number of CD3+CD56+ cells infused ranged from 1 to 16×10^6 (median value 5×10^6). Patients affected by solid tumors received in association to IL-1β or IL-2 a α-interferon. Protocol adherence was excellent and the toxicity profile was favourable. Only 2 patients developed low-grade fever during the first cycle of infusions (5%), recovered without antibiotic treatment. After CIK cells infusion, in patient’s peripheral blood the absolute median count of PBLS, CD3+, CD8+ and CD3+CD56+ cells significantly increased with a p-value of 0.034, 0.025, 0.054 and 0.038, respectively. Clinical outcome appeared promising. 2 of the 7 evaluable patients achieved complete response: 1 RCC and 1 HC and 2 patients had stabilization of disease (1 NHL and 1 RCC). At the last follow-up they are still alive at 26, 14, 19 and 9 months respectively, after the start of therapy. Conclusions. These preliminary data showed that adoptive immunotherapy with CIK cells is a safe therapy with some suggestion of efficacy that significantly enhances immune functions increasing absolute numbers of effector cells without side effects. If confirmed in larger scale studies, these promising results may have a favourable impact on conventional treatment strategy of malignancies.

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Methods. Blood samples were collected from 10 AML patients at diagnosis before induction chemotherapy and 5 healthy donors (control group). Mononuclear cells were isolated using gradient centrifugation with Ficoll-Paque and were differentiated into dendritic cells by culturing with 180 ng/mL calcium ionophore for 4 days. DC were stained for surface and intracellular ACE using two mAbs to ACE-1 (clone 1D8 for unfolded ACE (mainly located intracellular) and 9B9 for both native (surface) and unfolded ACE and further analyzed by flow cytometry. Results. LDC derived from AML blasts did not express surface ACE 1.5±0.9% of positive cells - clone 1D8 and 2.4±2% - clone 9B9. However, LDC contained a large amount of intracellular ACE: 67±16% (clone 1D8), 81.3±9% (clone 9B9). In contrast, surface ACE expression was revealed in 46.5±5.4% of donors DC (with mAb 9B9). DC derived from monocytes of healthy donors had lower intracellular ACE 10.3±9% of cells (with mAb 1D8) and 5.48±3.1% (with mAb 9B9). The proportion of intracellular ACE expressing LDC was significantly higher than in DC (p<0.001 and p<0.001 for clones 1D8 and 9B9, respectively). The proportion of surface ACE positive LDC was significantly lower than surface ACE positive DC (p<0.001 for clone 9B9). Conclusions. The data demonstrated the absence of ACE transport to the cell surface of LDC and therefore, provide another evidence of the distorted differentiation capacity of AML blasts.
of memory T cells. Aims. Based on these data, in this study we evaluated the ability of the adoptive transfer of the anti-leukemia response, in terms of specificity and phenotypic characteristics of CTLs, using common γ-chain cytokines (IL-2, IL-7 and IL-15) at different phases of the in vitro induction and expansion of anti-leukemia CTL lines. Methods. In particular, anti-leukemia CTL lines supported with the different cytokines were compared for i) levels of the in vitro proliferation and cytotoxic activity against target L6 and non-target parental cells, and ii) functional restoration of T memory and effector subsets. Results. We found that, even though sizeable levels of anti-leukemia T-cell response can be obtained in all cultures, the use of different cytokines during the various phases of the induction of anti-leukemia CTL response allows us to modulate not only the expanding the corresponding CTLs and their leukaemia-directed cytotoxicity, but also the percentage and the absolute number of T memory and effector cells, without loss of specificity. Conclusions. In particular, we demonstrated the crucial role of IL-15, in increasing T central memory (TCM) cells, potentially able to display long-term survival and capacity to in vivo proliferate in the presence of limited amount of L6. Further experiments are in progress to confirm this phenomenon and to evaluate precisely the role of IL-7 in the maintenance of TCM cells during the expansion of anti-leukemia CTLs.

0444

CROSS-COSTIMULATION BY DONOR ANTIGEN PRESENTING CELLS PLAYS A ROLE IN ACUTE XENENOCENE GRAFT-VERSUS-HOST DISEASE

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Background. Graft-versus-host disease (GvHD) limits the efficacy of allogeneic cellular immunotherapy for the cure of human disease. In mouse models, acute GvHD appears to be initiated by the encounter of donor T cells with host antigen presenting cells (APC). Cross-presentation of host antigens by donor APC arising de novo from the hemopoietic cell graft also participates to GvHD. Aim. To verify if the adoptive transfer of donor APC along with T cells plays a role in acute GvHD. Methods. Adoptive transfer of human T cells in conditioned non-obese diabetic (NOD)/severe combined immunodeficient (scid) and evaluation of the requirements for acute xenogeneic GvHD. Results. In vitro, human blood mononuclear cells (PBMC) proliferate in response to dendritic cells (DC) derived from NOD/scid mice. Proliferation depends on the presence of human APC. Posing costimulatory properties of human APC by chemical treatment inhibits proliferation, while neither blocking MHC class II-restricted antigen presentation with anti-human antibodies nor interferon γ-pulse providing the antigen-presenting cell, or addition of CD40-CD154-CD40L-expressing autologous DCs to GvHD results in the in vivo transfer of both virus-specific CD8+ and CD4+ T-cells is essential. Aims. In this study we developed a protocol for the generation of CMV pp65-specific CD8+ and CD4+ T-cell lines for adoptive transfer. The isolation and culture conditions were optimised to generate a suitable approach for clinical implementation which is fully compliable with Good Manufacturing Practice (GMP) conditions. Methods. PBMCs from five CMV seropositive donors were stimulated with different concentrations (6,6-66 µg/mL) of recombinant CMV pp65 protein (Milenyi Biotech) and/or HLA-A*0201/HLA-B*0702 restricted immunodominant pp65 peptides (NLV/TPR). Peptides used were clinical grade and recombinant protein was γ-irradiated (50 Kgy, 80°C) to eliminate possible microbial contamination. IFNγ producing cells were enriched using the IFNγ secretion assay (Milenyi Biotech) at day 3 after stimulation, and cultured with autologous feeders (10x) and low or high dose of IL-2 (10 or 50 IU IL-2/mL). At day 7-11 cells were harvested and cryopreserved. Cell lines were analysed at different time points for staining by peptide-MHC tetramer (NLV22/TPR-B7) and phenotypic markers. In addition, pp65-specificity was evaluated by intracellular IFNγ staining after restimulation with a pp65 protein-spanning pool of 15-mer peptides. CMV-specific lysis was tested in a 51-chromium release assay on pp65-transduced target cells. RESULTS. Enrichment of IFNγ producing cells after pp65 protein stimulation resulted in pp65-specific cell lines consisting of both CD8+ (median 28%, range 20-74%) and CD4+ T-cells (median 48%, range 12-79%). The CD8+ compartment contained immunodominant tetramer staining cells (median 60%, range 5-75%). The majority of both CD8+ and CD4+ T-cells produced IFNγ on restimulation with the pp65 peptide pool and cell lines showed CMV-specific lysis of target cells. The phenotype of pp65-specific T-cells was predominant CD28+/CD45RO+ and CD45RA+/CCR7+/CD62L−, although CCR7 and CD62L were transiently expressed at day 4 and 7 after stimulation. Addition of higher concentrations of protein during the initial stimulation had a negative effect on enrichment.
Probably due to non-specific stimulation of cells. Addition of immunodominant pp65 peptides resulted in stronger stimulation and proliferation of epitope-specific CD8+ T-cells, although isolation efficiency was not increased. Except for the enhancement of proliferation, no effect of high dose compared to low dose IL-2 was observed. Cryopreservation did not affect the composition or functionality of T-cell lines. Summary/Conclusions. Based on these results we generated a GMP-proven method for generation of pp65-specific T-cell lines using 6.6 μg/mL of pp65 protein for stimulation followed by isolation of specific T-cells based on IFNγ production. Isolated T-cells will be cultured for a short period on low dose IL-2 in order to maintain maximal in vivo potential. This procedure yields GMP-grade T-cell lines comprising both CD8+ and CD4+ CMV-specific T-cells, which will be assessed for their clinical efficacy.

0446 FUNCTIONAL CHARACTERIZATION OF CYTOMEGALOVIRUS (CMV)-SPECIFIC CD4 AND CD8 T CELL LINE GENERATES BY USING PROTEIN-SPANNING POOLS OF PP65 AND IE1 DERIVED PEPTIDES
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Background and Aims. Reactivation of latent CMV in immunocompromised recipients of allogeneic stem cell transplantation remains a major cause of morbidity and mortality. Reconstitution of immunity by CMV-specific immunotherapy is an attractive alternative to standard treatment. In this study, we have analyzed the functional properties of CMV-specific T cells freshly isolated from donors for the treatment of CMV-infected recipients. Results. CD4 and CD8 CMV-specific T-cell lines were successfully expanded from 10 CMV seropositive donors. Cultured T cells expressed CD8 (mean 70%, range 60-81%) and CD4 (mean 20%, range 15-26%) and showed an Effector Memory (mean 26%, range 19-30%) or an Effector Memory RA-Positive phenotype (mean 67%, range 59-77%). An enriched CMV-specific T-cell population was observed after pentamers staining (7-45% pentamer-positive T cells). In all cases, cultured T cells showed a cytolytic activity against CD8-peptide pulsed target cells (average lysis 50%, range 40-55%) and, to a lesser extent, against CD4-peptide pulsed target cells (average lysis 35%, range 30-40%). In addition, cultured T lymphocytes were able to proliferate and to produce intracytoplasmic IFN-γ (average production 50%, range 35-60%) after exposure to peptide-pulsed DC. CMV-specific T cells were also analysed for the expression of adhesion molecules and chemokine receptors and for their ability to migrate in response to inflammatory (CXCL9, CCL3 and CCL5) and constitutive (CXCL12) chemokines. T cells showed high levels of CXCRI3 (average expression 94%, range 81-99%), CCR1 (average expression 61%, range 57-92%), and to a lesser extent, CCR4 (mean 25%, range 10-61%). In accordance with this profile, cultured T cells strongly migrated in response to CXCL12 (mean Migration Index (MI) 1.8, range 1-5.2), CCL5 (mean MI 7.2, range 5.4-11), CCL3 (mean MI 2.3, range 1.3-4.2) and CXCL9 (mean MI 2.8, range 1.5-2.7), which are involved in the recruitment of effector cells to peripheral sites of viral infection. Finally, CMV-specific T cells showed high level of CD49d (≥ 98%), which guides the homing of effector cells into inflamed tissues, and low levels of CD62L, a molecule involved in the migration to lymphoid organs. Conclusions. In conclusion, we demonstrated the possibility to generate activated and armed CMV-specific T-cell lines, potentially able to reach viral-infected tissues and to recognize and kill CMV-infected cells.

0447 MICROBEADS AS ARTIFICIAL APCS AND CELL BRIDGES FOR T CELL CANCER THERAPY
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Cancer results from genetic alterations within tumor cells that promote their proliferation and resistance to apoptosis, but also to the failure of the immune system in the destruction of the tumor cells. Adoptive T cell therapy is achieving its first irrefutable successes in the reprogramming of immune responses against tumor cells. These successes are based on the grafting of anti-tumor T lymphocytes to hosts previously lymphodepleleted by chemotherapy. We are attempting to improve this therapeutic strategy, because is the most common leukemia and is usually treated with lymphodepleptive therapy. Furthermore, we and other have demonstrated that in these patients there is a reactive expansion of antileukemic T-cells which maintain certain control over the growth of the leukemic cells. We have developed flow cytometry methods for the study of in vitro interactions between T cells and leukemic cells. These methods allow the accurate measurement of the growth and apoptosis of leukemic cells in coculture with T lymphocytes. Our laboratory is one of the pioneers in the use of microbeads coated with monoclonal antibodies (MABs) in the polyclonal stimulation of T lymphocytes in vitro. Microbeads coated with Abs combinations can simultaneously stimulate the T cell antigen receptor and costimulatory receptors such as CD28 working like artificial antigen presenting cells that can be used to activate and expand antileukemic T cell clones. We are developing in vitro methodologies to break the immune tolerance to the leukemic B-CLL cells and to induce the cytotoxic T cell response against these tumor cells. The first strategy is to polyclonally stimulate (with anti-CD3, anti-CD28 and IL-2) T lymphocytes from these patients. A second and very promising strategy was pioneered by our laboratory. It uses microbeads to bridge leukemic cells and T cells. It uses one antibody against a leukemic cell antigen and other against costimulatory antibodies of cytotoxic lymphocytes (anti-CD28). These microbeads link the leukemic cell and the cytotoxic T lymphocyte providing costimulatory signals for the T cell in the case it recognizes one antigen presented by the leukemic B cell. The application of methodologies of cell enumeration in coculture has demonstrated the efficacy of anti-CD28 and anti-CD28 coated microbeads in the stimulation of cytotoxic T cells to kill autologous leukemic B-CLL cells. We are now developing methods of generation of antileukemic effector T cells in vitro. We have demonstrated the efficacy of these cytotoxic T cells to kill autologous leukemic B cells in vitro. We also want to identify and select the T cell subsets with the highest potential for growth and the strongest killing activity against leukemic cells.

0448 THE IMMUNOMODULATORY EFFECTS OF HUMAN UNRESTRICTED SOMATIC STEM CELLS ON CORD BLOOD T CELLS
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Background. unrestricted somatic stem cells (USSCs) generation was initiated from fresh and cryopreserved cord blood. Reports are indicating that USSCs have unique immunologic properties, making them ideal for cellular therapies. USSCs are not immunogenic, they do not stimulate allore cognition, and they cause little allore cognition, and they cause little activation, and they escape lysis by cytotoxic T-cells and natural killer (NK) cells. Thus, USSCs may be transplantable between HLA-mismatched individuals without the need for host immunosuppression. Aims. To evaluate probable immunomodulatory effects of USSCs on T cells proliferation. Methods. USSCs were plated in 96-well plates (2,000/well), and co-cultured for 3 days with T cells isolated from cord blood. In control group, cord blood T cells did not co-culture with USSCs. After cord blood T cells stimulated by PHA for 60 hours, T cell proliferation was assessed by MTT assay. Secretion of IFN-γ from stimulated cells was measured by ELISA kit. Expression of immunoregulatory molecules on USSCs was analyzed by flow cytometry. Results. USSCs expressed major histocompatibility complex (MHC) class I, lymphocyte function-associated antigen (LFA-3) constitutively and intercellular adhesion molecule (ICAM-1) antigens upon γ interferon treatment but do not express CD80, CD86, or CD40 costimulatory molecules. The results from IFN-γ measurement showed that cord blood T cell proliferation was suppressed when 2,000 USSC were plated on each well. Summary/Conclusions. USSCs actively inhibit T-cell proliferation, suggesting that allogeneic USSCs transplantation might be accomplished without the need for significant host immunosuppression. USSCs transplantation may be used for modulation of immune system in hyper reactive and autoimmune diseases.
**Myelodysplastic syndromes**

**0449 VALIDATION OF THE NIJMEGEN PREDICTIVE SCORE FOR INDUCTION THERAPY IN MDS WITH HIGH-RISK MDS OR AML**


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**Background.** Intensive Chemotherapy in high-risk MDS patients still is a matter of debate. The majority of MDS patients is too old to undergo induction chemotherapy, the relapse rate is high and the proportion of long-term survivors is with about 10-25% relatively low. Taking into account comorbidities, side effects and complications of the therapy as well as about 10% early death rates, one should try to select patients very carefully for intensive chemotherapy. Although it became clear that the initial karyotype predicts CR rate as well as long-term outcome (Knipp et al Blood, abstract 2004), it is not possible to use this as the only parameter to decide whether or not the patient should undergo induction therapy. The Nijmegen group recently proposed a predictive score that was calculated on the basis of a large amount of patients (Ottenveld, Leukemia Research, abstract 2005). Besides the karyotype, WBC, age, an-incident haematological malignancy and number of cytopenias were rated differentially to form 3 risk groups, associated with different long-term outcome. Aims. In order to validate this predictive score with an independent dataset, we investigated 129 patients. Methods. There were 283 patients with either high-risk MDS or MDS/AML or AML who were treated with induction at our institution between 1988 and 2005. Median age was 57 years (16-74). The patients received induction therapy with Ara-C and an Anthracyclin and patients younger than 60 years additionally received Etoposide. 16 patients underwent autolografting after achievement of CR. 58% of the patients entered CR, 9% of the patients achieved PR, 23% of the patients had no remission and 10% of the patients died within 5 weeks after induction. Results. We then retrospectively tested the Nijmegen Score using this database. 25 patients were allocated to the low risk group (9%), 129 patients to the intermediate risk group (45%) and 129 patients to the high-risk group (46%). There was a difference in early death rate (0% vs. 6% vs. 15%, p=0.002). The overall survival was 40 months in the low, 24 month in the intermediate and 12 months in the high-risk group (p<0.00005). The difference between intermediate and high-risk group was also statistically significant (p<0.00005). The percentage of patients still alive two years after induction was 75% vs. 48% vs. 27%, and after 5 years 50% vs. 25% vs. 7%. Only within the low-risk group, there are patients with a long-term survival up to 16 years. Conclusions. These data indicate that a) the Nijmegen Score in validated in a large independent patient group, b) the risk groups are well defined by a number of specific response patterns. Knowing these characteristics will enable the physician to safely induce remissions in an important part of patients.

**0450 LENALIDOMIDE IN DEL(5q) MDS PATIENTS: DIFFERENT PATTERNS OF RESPONSE**

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The novel amino-substituted thalidomide analogue lenalidomide (Revlimid®) has recently been approved in the USA for the treatment of transfusion dependent anaemia due to low- or intermediate-1-risk myelodysplastic syndromes associated with a del(5q) cytogenetic abnormality or with additional chromosomal abnormalities. The decision was based on data of a phase II study on a total of 148 del(5q) patients that showed an impressive number of cytogenetic remissions and transfusion independence. Given that 15% of patients with MDS bear the del(5q) abnormality, and that 12,000 to 15,000 new MDS cases are diagnosed yearly in the European Union, up to 2,300 new patients every year will be eligible for treatment with lenalidomide. In this presentation, we give an overview of treatment approaches based on our experience gained on more than 40 patients treated with lenalidomide at a single center. An important number of patients have different patterns of response to the drug the attending physician must be familiar with to avoid under- or overtreatment and to assure the best possible care. Five response types are described: First, the uncomplicated responder who does not need drug, dose reduction and goes into long-term hematological and cytogenetic remission. Second, the typical responder who does require dose reduction but still achieves long-term remission. Third, the intermittent responder needs careful long-term blood count monitoring and individual dosing. This type of responder is characterized by an initial beautiful response with both transfusion independence and cytogenetic response that suddenly vanishes and leads to recurrence of initial transfusion dependence. Some of these patients benefit from a simple drug holiday, some from resuming lenalidomide after a varying drug interruption. Fourth, there are very few late responders who achieve transfusion independence weeks after stopping the drug for lack of efficiency. Finally, some patients simply do not respond to the drug without us being able to identify predictive factors which patients belong to this group. It is interesting to note that cytogenetic remission is not a prerequisite for long-term response. Some patients remain del(5q) positive with a long-term ongoing hematologic remission. On the other hand, we will also report our experience in patients with complex cytogenetic aberrations who seem to do as well as 5q-syndrome patients. Regarding adverse events, we will present our experience in treating skin reactions, pruritus and scalp itching, muscle cramps, diarrhea, hypothyroidism, and of course neutropenia and thrombocytopenia. None of our patients required platelet transfusions because we regularly interrupted treatment at values of <50,000/µl. Grade 3 neutropenia is common, but only the minority of patients needed granulocyte stimulating cytokines. Titrating the drug until neutropenia and thrombocytopenia occurs has proven effective in achieving erythroid response during regeneration of haematopoiesis. We conclude that lenalidomide is a reasonably safe drug in the del(5q) patient population that is characterized by a number of specific response patterns. Knowing these characteristics will enable the physician to safely induce remissions in an important part of patients.
those induced by alkylation agents. Since combining 2 alkylating agents causes a very high risk of MDS/tAML, the combination of fludarabine and cyclophosphamide may be highly leukemogenic. Our data support this hypothesis. The recognition of 50% of our cases retrospectively, is in line with emerging trial data which suggest that tMDS/tAML patients who had non-tMDS/tAML POLYMERISATION recently described that CD34+ cells from patients with myelodysplasia diversity Medical Center Utrecht, UTRECHT, Netherlands patients who had migration of CD34+ cells from low risk MDS patients.

To investigate the role of Rac in the RAC-actin pathway, both of these pathways appear to be impaired in MDS CD34+ cells, resulting in a decreased SDF-1-induced migration.

Table 1. Comparison between patients groups who did and did not develop tMDS/tAML.

Patient group Median age in years Median dose F mg/m² (range) Median dose H (range) vs F dose (tMDS/tAML vs non-tMDS/tAML) Significance vs H dose p value for difference between F dose (tMDS/tAML vs non-tMDS/tAML) vs other chemotherapy given (range) Median no of other chemotherapy given (range)

tMDS/tAML patients (n=73) 73 (62-82) 541 (350-703) 375 (420-480) 2 (1-3) 2 (0.01) 2 (0.4)

Non-tMDS/tAML patients (n=33) 72 (42-83) 300 (64-1038) 0.005 0.01 0.005

Subgroup (n=69) 69 (46-83) 270 (64-1038) 0.005

Subgroup (n=72) 72 (42-79) 375 (100-690) 0.005

1 patient had fludarabine, mitoxantrone and dexamethasone.

0452 IMPAIRED SDF-1-INDUCED MIGRATION OF CD34+ CELLS FROM MDS PATIENTS AS A RESULT OF DECREASED ACTIVATION OF PROTEIN KINASE B, RAC AND F-ACTIN POLYMERISATION

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Background. SDF-1 is a chemotactic agent that is implicated in mobilisation, retention and homing of haematopoietic stem cells. It was recently described that CD34+ cells from patients with myelodysplasia (MDS) demonstrate an impaired SDF-1-mediated migration, despite the presence of high levels of SDF-1 in the bone marrow. Aims: To investigate the molecular mechanisms underlying the decreased SDF-1-induced migration of CD34+ cells from low risk MDS patients. Methods: Migratory behaviour of sorted CD34+ cells was assessed using Transwell assays. Phosphorylation status of protein kinase B (PKB) and extracellular signal-regulated kinase (ERK1/2) were analyzed by Western blot analysis. Rac activation was studied by performing pull down assays. Results: We confirmed the reduced migratory capacity of MDS progenitors towards SDF1 compared to normal CD34+ cells (5.7±2 vs 18±2%, n=7, p=0.02). This defect could not be attributed to lower SDF-1-receptor expression, as the number of CXCR4-expressing cells was similar between MDS and normal CD34+ cells (16%±3 vs 16%±1, n=6, p=0.5). It has been described that the rate of F-actin polymerisation might be limiting for cell migration. We therefore examined F-actin polymerisation in response to SDF-1 and showed that this was significantly reduced in MDS CD34+ cells compared to normal CD34+ cells (151±5 vs 176±8, n=6, p=0.03). To further elucidate the molecular mechanisms of the impaired migration we investigated the involvement of the phosphatidylinositol 3 kinase (PI3K), ERK1/2 and Rac signalling pathways in migration and actin polymerisation of normal CD34+ cells. Pre-treatment of CD34+ cells with the ERK inhibitor U0126 lead to significant decrease in migration (21%), as did inhibition of PI3K pathway with LY294002 (50%). Incubation of CD34+ cells with the Rac inhibitor NSC23766 abrogated SDF-1-induced migration consistently (61%). However, U0126 and LY294002 treatment did not affect the SDF-1-induced F-actin polymerisation of CD34+ cells, whereas incubation of CD34+ cells with NSC23766 did attenuate actin assembly in response to SDF-1 (191±16 vs 137±6, p=0.04). We subsequently questioned whether the disturbed migration of MDS CD34+ cells is in part the result of an ineffective activation of one of these signalling pathways. Investigation of phosphorylation status of ERK1/2 and the PI3K target PKB in response to SDF1 showed that ERK1/2 activation was decreased in 3 out of 6 patients, whereas PKB activation was impaired in 5 out of 6 patients when compared to normal CD34+ cells. Furthermore, levels of Rac-GTP, amount of Rac present in the lysates, were lower in SDF-1 stimulated progenitors from MDS patients compared to healthy controls (n=3). Conclusion. These results indicate that although ERK1/2, PI3K and Rac are involved in SDF-1+chemotactic gradient, only Rac is necessary for F-actin polymerisation in response to SDF-1. It is conceivable that at least two separate pathways are required for migration, and at least a PI3K-dependent pathway and an independent Rac-actin pathway. Both of these pathways appear to be impaired in MDS CD34+ cells, resulting in a decreased SDF-1-induced migration.

0453 EVI-1 GENE EXPRESSION IN MYELODYSPLASTIC SYNDROMES: QUANTITATIVE ASSESSMENT AND IN VITRO MODULATION INDUCED BY ARSENIC TRIOXIDE

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Background. The EVI-1 gene is located in chromosome 3q26 and codes for a zinc finger protein which acts as transcriptional repressor. Rearrangements of the EVI-1 locus in chromosome band 3q26 are associated with poor prognosis in myeloid malignancies. The overexpression of the gene has been described in a subset of patients without evidence of gene rearrangements. Moreover it was suggested that the overexpression of the gene confers a high degree of sensitivity to Arsenic trioxide therapy. Aims. The aim of the study was to analyze the expression level of EVI1 in a large number of different subtypes of myelodysplastic syndromes and to study the in vitro effects of arsenic trioxide. Methods. We analyzed the expression levels of EVI-1 in 250 BM samples and 162 FB collected from 345 MDS patients. 126 were refractory anaemias (RA), 144 refractory anaemias with excess of blasts (RAEB) and 75 RAEB-T or secondary AML (s-AML). Moreover we tested 37 de novo AML patients and 22 BM and 35 PB samples obtained from healthy volunteers. 4 AML and 1 MDS patients showed the 3q26 rearrangement detected by cytogenetic analysis. The expression level of EVI-1 was established using quantitative Real-Time PCR based on a specific primer and probe set (Assays-on-Demand, Applied Biosystems). The values obtained were normalized using ABL as housekeeping gene and the final results were expressed using the mean value of 2-DeltaDeltaCt = range 0-11) and undetectable levels in PB. By contrast, in 134 patients over 345 (39%) abnormal levels of EVI-1 were detected. Significantly higher levels were found in patients with 3q26 rearrangements (range 294-35120). The patients expressing high levels of EVI-1 were distributed as follow: 38 RA (mean value of Delta Delta Ct = 49; range 11-64), 62 RAEB ( mean value = 128; range 60-264) and 34 RAEB-T (mean = 2196; range 162-6653). 5 out of 37 de novo AML showed abnormal expression of EVI-1 (mean value 468 range 56-580). EVI-1 expression was evaluated during follow-up of twelve patients who converted into overt leukaemia and in all the cases EVI-1 levels increased during progression. In vitro treatment with arsenic trioxide induces in 22 out of 30 samples (28%) a significant increase of BFU-E colony number, and this was observed mainly in patients characterized by high EVI-1 levels (17 out of 22). Moreover a significant reduction of EVI-1 gene expression was observed after arsenic trioxide incubation (p=0.005) as compared to controls. Conclusions: These data allow to establish that the overexpression of EVI-1 is present in 39% of MDS patients regardless of the presence of the 3q26 rearrangement. The overexpression seems to be more frequent in RAEB and s-AML respect to RA and it increases during disease progression. The arsenic trioxide treatment induces reduction of EVI-1 transcript amount and a significant increase of BFU-E growth in patients overexpressing EVI-1.

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CYTGENETIC EVOLUTION IN DEL(5q) MYELODYSPLASTIC SYNDROME

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Myelodysplastic syndromes are clonal hematopoietic stem cell disorders that are characterized by a wide prognostic heterogeneity. While some MDS like the pure sideroblastic anemia tend to remain stable for years, others evolve to higher risk MDS or acute myeloid leukemia. During disease progression, cytogenetic evolution occurs and is an independent prognostic risk factor. To assess cytogenetic evolution in MDS with del(5q), we analyzed in data of 33 patients with a novo del(5q) MDS. Inclusion criteria were del(5q)/11.1 MDS at initial cytogenetic investigation in at least two metaphases irrespective of International Prognostic Scoring System (IPSS) rating. Cytogenetic evolution was defined as appearance of additional chromosomal abnormalities within the initial del(5q) clone. Gain of chromosomes was accepted if recorded in at least two metaphases, loss of chromosomal material had to be evident in at least three metaphases according to ISCN rules, or if single cell abnormalities were confirmed by FISH analyses. Additional clones with chromosomal aberrations other than del(5q) were regarded as unrelated clones but not as cytogenetic evolution within the del(5q) clone. Initial cytogenetics were performed between 1989 and 2003. The last follow-up investigation was done in 2004 by the reference cytogenetic center of the German MDS study group (BS). Median age of the 33 patients was 62.2 years (range, 32 to 83). All patients had at least two cytogenetic evaluations (range, 2 to 9) with a median time between first and last examinations of 36 months (range, 3 to 172). 19 patients had RA according to FAB, 4 had RARS, 8 RAEB, and 2 were not classifiable. 2 patients (6%) acquired additional cytogenetic lesions within the initial del(5q) clone. These were t(1;3)(p33;p14) after 25 months, and inv(3)(q13q25) after 48 months. One patient had a trisomy 8 in a different clone in a previous examination in 8 metaphases that was inapparent at follow-up in 20 months after 32 months. One patient with RAEB who had initially additional trisome 3 in the del(5q) clone acquired a second clone with a der(18;21). All true cytogenetic evolutions occurred in 5q-syndrome patients. One of the two patients with cytogenetic evolution did not respond to lenalidomide treatment. However, karyotype complexity in del(5q) MDS does not seem to impact on response to lenalidomide therapy as there is increasing evidence that del(5q) patients with complex karyotype have the same amount of cytogenetic recurrence rates as 5q-syndrome patients. As a conclusion, del(5q) MDS display long-term clonal stability, with cytogenetic evolution being evidenced in less than 10% of patients. Clonal evolution is not necessarily linked to advanced MDS at initial presentation.

A ROLE FOR THE ENDOPHASIC RETICULUM AND THE MITOCHONDRION IN ERYTHROID CELL APOPTOSIS THAT CHARACTERISES LOW GRADE MYELODYSPLASTIC SYNDROMES

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Cell death by apoptosis was shown to account for the ineffective erythropoiesis that characterises low grade myelodysplastic syndromes (MDS). We have shown previously that the death receptor Fas was overexpressed at the surface of MDS erythroid precursors. Using an ex vivo liquid culture to analyse the differentiation of low grade MDS CD34+ cells into red cells, we demonstrated that apoptosis of MDS erythroid precursors could be prevented by either Fas-Fc or the ectopic expression of a dominant negative mutant of the adapter molecule FADD (Fas-associated death domain), a component of the death-inducing signalling complex, which mediates death after death receptor stimulation. We and others also showed that the release of mitochondrial cytochrome c participated to this process, suggesting a connection of the extrinsic to the intrinsic pathway of apoptosis. To further address this latter question, we over-expressed the anti-apoptotic Bcl-2 protein in CD34+ bone-marrow cells before inducing their erythroid differentiation. For that purpose, we used lentiviral constructs including CDNA encoding either wild-type Flag-Bcl-2 (WT) or a Flag-Bcl-2 targeted to endoplasmic reticulum (ER) by a cytochrome b6 sequence. These constructs, which also encodedGFp under the control of an IRES, were used to infect either control (n = 10) or low grade MDS (n = 15) CD34+ bone-marrow cells. These cells were subsequently selected in G418, cultured in methylcellulose, and stained for apoptosis. As expected, we demonstrated that apoptosis in CD34+ cells present in the WT infected cultures was significantly lower than in control and MDS erythroid precursors, compared to less than 10% of those infected with the control vector. Specific targeting of Bcl-2 to the ER was confirmed by both immunofluorescence analyses and the lack of inhibition of lonidamide-induced cell death. Overexpression of Bcl-2 WT which is located in mitochondria and ER, or overexpression of Bcl-2 ER delayed the erythroid differentiation of both MDS and normal cells. In contrast, the two Bcl-2 encoding viruses specifically inhibited phosphatidyserine externalisation, MMP decrease and caspase-9 and -3 activation associated with erythroid differentiation of MDS bone-mar-
row CD34+ cells. Interestingly, over-expressed Bcl-2, either wild-type or ER-targeted, failed to affect the truncation of the BH3-only protein BID. This suggests that the mitochondrial pathway of apoptosis is activated downstream of Fas through BID. Over-expressed Bc-2 reduced also the release of ER calcium in response to thapsigargin, reflecting lower intracellular calcium stores and a less apoptosis. Altogether, these results confirm the involvement of mitochondria in erythroid cell death that characterises low grade MDS and strongly argue for a participation of ER in this apoptotic pathway, both organelles acting downstream of the death receptors.

0457
ISSUES AFFECTING QUALITY-OF-LIFE IN PATIENTS LIVING WITH MYELODYSPLASTIC SYNDROMES: RESULTS OF PATIENT FORUM DISCUSSIONS IN EUROPE

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Background. Patients living with MDS experience significant deterioration in their quality-of-life. New treatments (approved and under development) for MDS have provided patients with hope that this deterioration in quality-of-life can be limited or eliminated in the near future. Aims. Patient forum discussions were conducted with the goals of determining the key issues regarding quality-of-life (QoL) in patients living with MDS including: feelings about the attitude of, and support offered, by health care providers, the patient’s depth of knowledge about MDS, and effect of treatment on QoL. Educational programming will be developed based on the information derived from these forums. Methods. Nine MDS Foundation Centers of Excellence volunteered to participate and 4 forums convened to date. Questionnaires were developed, vetted, and translated by participating sites. Questionnaires were consistent in all locations. Discussion focus varied due to the free-lying nature of the forums. These forums were conducted in Edinburgh, Paris, Bournemouth (England), and London. Results. A total of 67 patients and 92 caregivers participated. Patient sample was Caucasian (100%); male (52%); female (48%); Age range <50 (10%), 50-75 (57%), >75 (33%). 5 have less than 6 years of education, 30 had 10-12 years, and 32 had >12 years. 50 are married (75%) and the 95% live with other people. 21% are employed full or part time and 50% are retired with 29% unknown. Patient QoL experiences were similar between sites and reflected substantial feelings of life disruption due to MDS and time required for disease management. Physician visits, testing, transfusion, treatment, travel time, diagnosis/adverse event management contributed to feelings of lose of life control. Fatigue is the issue affecting QoL most often - impacting patient’s ability to perform activities of daily living, work, and participation in social and family life. Emotional well being is significantly decreased and described as waiting for something to happen. Physician relationships at the COEs were viewed positively by the majority while relationships with community physicians were viewed in a negative context due to physician’s lack of knowledge. Patients described time spent educating the doctor. Nurses were viewed as key to patient’s knowledge and well being. Patients expressed overall satisfaction with current treatment however 65% felt that new drugs were not being made available quickly enough within the EU. Transfusions, in tandem with chelation therapy, were viewed as impacting QoL significantly second only to fatigue. Patients viewed transfusions as a necessary evil to deal with their fatigue. Caregivers expressed a need for information to assist them in dealing with family/friends with MDS. Conclusion. MDS has a substantial impact on patients QoL including interactions with family and friends. Physicians and other healthcare professionals should be aware of this impact and attempt to provide patients with information and options to lessen the burden of this disease and minimize its impact. New treatment options should be explored with patients, including participation in clinical trials, with the goal of improving QoL and lessening fatigue, oral medications for chelation therapy.

0458
FOUR DIFFERENT TYPES OF MDS PATIENTS WITH 5Q- ANOMALIES

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Background. About 50% of all MDS patients show karyotype aberrations at the time of diagnosis. The most frequent chromosomal anomaly is the del(5q) aberration. The WHO re-recognized the 5q- Syndrome as a separate entity within the group of myelodysplastic dis-or-ders. However, a high number of MDS patients show 5q- aberrations together with another abnormality (such as a complex karyotype). Aim. A better description of the different types of 5q- aberrations in warranted. Methods. We screened the German MDS registry Düsseldorf for patients presenting with 5q- aberrations, regardless of WHO type and other chromosomal aberrations. Results. Out of 2979 patients in the registry, 1068 patients have been karyotyped at diagnosis (56%). 180 of them (17%) showed a 5q- anomaly either alone or in combination with other aberrations. We then separated the patients into 4 groups: 5q- Syndrome (del(5q) as a single anomaly, medullary blast count <4, Group A), del(5q) as a single anomaly with elevated blasts (Group B), del(5q)-MDS within a complex karyotype (group D). We then examined haematological data and prognosis of the 4 groups. For calculating the prognosis, patients who underwent Induction therapy, allogeneic stem cell transplantation or Revlimid treatment were censored. Per definition, all patients in group A had a medullary blast count of <5%, and all patients in group B had RAEB or RAEB-T. Group C consisted of RA and RARS in 85%. 66% of the patients in group D had RAEB or RAEB-T. The degree of hematopoietic insufficiency was more pronounced from in B, C and D and the prognosis was adverse in group B, C and D. Median survival of group A was 65 months as compared to 68, 51 and 7 months respectively (p<0.005). The cumulative risk of AML was 13% in the group A, as compared to 33%, 42% and 55% (p<0.05). Conclusions. These data show that the prognostic impact of 5q-anomaly as well as its pathophysiological impact is heavily influenced by other factors, such as medullary blast count and additional aberrations. This should be taken into account, when assessing the prognosis is planning treatment for those patients.

0459
THE PROGNOSTIC MEANING OF INVOLVEMENT OF 5Q- ABERRATIONS IN PATIENTS WITH MDS AND COMPLEX KARYOTYPE

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Background. It is well known, that patients with Myelodysplastic syndromes with a complex karyotype have a very poor prognosis, facing a median survival of less than 1 year. There is ample evidence on the efficacy of lenalidomide in patients with del(5q), not only in 5q- Syndrome but also in patients with del(5q) as part of a complex karyotype. Aims. In order to examine the prognostic role of del(5q) involvement within complex karyotype types, we screened the German MDS registry Düsseldorf. Methods. A complex karyotype was defined according to the IPSS definitions. 155 patients were diagnosed with a complex karyotype regardless of WHO type and other chromosomal aberrations. Results. We then separated the patients into a group with involvement of del(5q) (n=58) and a group, in which del(5q) was not part of the complex karyotype (n=102). The distribution of both groups to WHO types did not differ significantly. Clinical and hematological characteristics (haemoglobin, platelet count, WBC, ANC, LDH) were not different between the groups; median age at diagnosis was 63 years in the non-del(5q) group and 66 years in the del(5q) group. The median survival of the del(5q) group was 7 months as compared to 14 months in the patients without 5q-involvement (p=0.006). 12 months after diagnosis, 22% of the del(5q) patients alive as compared to 49% of the non-del(5q) patients. 77% of the del(5q)-pa-tients and 75% of the non del(5q)}
patients died disease-related (AML, infections, bleeding). The risk of AML 12 months after diagnosis was 60 and 54% in the del(5q)- group and 44 and 50% in the non del(5q) group (p=n.s.). The overall percentage of patients that developed AML was not different (53% vs 55%, p=n.s.). Conclusions. Del(5q) is associated with an extreme poor prognosis when diagnosed within a complex karyotype. Because only a minority of these patients can undergo a curative ap-proach, the efficacy of lenalidomide should be studied in these patients group the near future.

0460

MRI T2* MEASUREMENTS SHOW NO MYOCARDIAL IRON LOADING IN PATIENTS WITH MYELODYSPLASIA ON LONG-TERM BLOOD TRANSFUSION THERAPY

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Background. Iron overload is inevitable in chronic transfusion-depend-ent anemia. Excess iron can accumulate in the liver, endocrine organs and heart which may result in end-organ damage. Mortality from iron over- load in thalassaemia is dependent on the magnitude of myocardial iron load. It is standard practice to initiate iron chelation therapy early in children with chronic transfusion-dependent anemia because it improves their life expectancy. Myelodysplastic syndromes (MDS) also result in chronic transfusion-dependent anemia but affects older adults. However, there is no data showing improved life expectancy with iron chelation therapy in this group of older patients with significant co-morbidities. Accurate measurement of iron overload requires tissue biopsy which carries a high risk of major bleeding in patients with myelodysplastic syndromes. Serum ferritin is an indirect surrogate marker for iron overload but lacks precision and reliability. MRI T2* measures iron load in liver and myocardium. The accuracy and reliability of this method has been validated for hepatic and myocardial iron load. Aims. 1. Assess hepatic and myocardial iron load using MRI T2* in chronic transfusion-dependent good-prognostic MDS patients. 2. Correlate hepatic and myocardial iron loading to the duration of transfusion therapy, serum ferritin levels, and iron chelation therapy. 3. Correlate cardiac function to iron load. Methods. Good prognostic MDS patients on long-term trans- fusion therapy were identified using IPSS scoring system. Liver function measurements in all patients. Hepatic and myocardial iron load was classified as none, mild, moderate and severe based on MRI T2* values (see Table 1).

Results. 1. Myocardial iron overload was not detected by MRI T2* in patients with serum ferritin greater than 1000 mcg/L. Hepatic and myocardial iron load was classified as none, mild, moderate and severe based on MRI T2* values (see Table 1).

Cardiac function assessment included measurement of left ventricu-lar ejection fraction (LVEF) using MRI. We report the results obtained in 7 patients who have completed the study. The median number of red cell units transfused was 106 units (range 42-257 units). The median duration of blood transfusions was 3 years (range 2-12 years). The medi-an serum ferritin level at the time of study was 4400 mcg/L (1560-6651 mcg/L). Six patients were receiving iron chelation therapy at the time of study. None of the 7 patients had clinical features of hepatic insufficien-cy at the time of the study. Results. 1. Moderate hepatic iron overload was detected by MRI T2* measurements in all patients on chronic transfusion therapy. 2. Raised serum ferritin levels correlated with hepatic iron overload. 3. Myocardial iron overload was absent by MRI T2* measure-ments in all patients. 4. Cardiac LVEF was normal in all patients. Conclu-sions. 1. Myocardial iron overload was not detected by MRI T2* in multi-transfused good prognostic MDS patients. 2. Myocardial iron load did not correlate with hepatic iron load or serum ferritin level. 3. Larger studies are needed to clarify these findings and to determine the role of iron chelation therapy in these patients.

0461

EVIDENCE FOR A ROLE OF CD40 IN THE PATHOGENESIS OF LOW-RISK MYELODYSPLASTIC SYNDROMES

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Background and aim of the study. Myelodysplastic syndromes (MDS) form a heterogeneous group of clonal haematopoietic disorders charac-terized by peripheral cytopenia, marrow dysplasia and an increased risk to develop AML. There is increasing evidence that the cytopenias in early phase MDS are partially caused by immunological mechanisms. The aim of this study was to investigate if the CD40-CD40L interaction plays a role in the pathogenesis of MDS-related bone marrow failure. Our hypothesis is based upon the knowledge that the interaction between CD40 and its natural ligand CD40L (CD154) is involved in normal immune responses, but also in the pathogenesis of several non-hematological disorders. Methods. 1/ With FACS we measured the expression levels of CD40 on CD14+ monocytes and CD40L on CD4+ blood mononuclear cells (PBMCs) and controls. 2/ CD14+ cells were isolated from PB from 17 patients (14 RA, 2 RARS, 1 RAEB) and 19 controls with MACS columns and cultured for 7 days in IMDM + 15% fetal bovine serum. They were subsequently stimulated for 24h with lipopolysaccharide (LPS) 1.0 μg/mL or agonist monoclonal anti CD40 antibody (clone 64, Bioceros NV, Netherlands) at a concentration of 10 μg/mL. TNF-α concentrations in supernatants were measured with ELISA. 3/ Bone marrow MNCs of 11 patients (5 RA, 2 RARS, 3 RAEB and 1 RAEB-t) were cultured in methylcellulose + growth-factors (M4454, Stem Cell Technologies) in the presence or absence of 10 μg/mL 5D12 (antagonist chimeric monoclonal anti-human CD40 antibody, Bioceros NV, The Netherlands). Results. 1/ MDS patients had a significantly high-er percentage of circulating CD40+ CD14+ (9.40% ± 2.05 vs. 1.89% ± 0.55, p=0.0125), and CD40L+/CD4+ cells (2.65 ± 2.76 vs. 5.99 ± 1.34, p=0.049) compared to controls. 2/ CD40-ligation, but not LPS, induced a significantly higher TNF-α production in patients compared to controls (588 ± 516 vs. 83 ± 24 pg/ml, p=0.0065). In patients, TNF-α production after CD40 stimulation was also significantly higher than after stimulation with LPS (588 ± 516 vs. 64 ± 189 pg/ml, p=0.016). In controls, TNF-α levels after LPS or CD40 stimulation were comparable. 3/ Co-culture of MDS bone marrow MNCs with 5D12 increased in vitro colony formation (141 ± 48 vs. 116 ± 43, p=0.067), an effect not observed in controls. Conclusion. We conclude from these observations that CD40-CD40L interactions might play a role in the pathogenesis of MDS-related bone marrow failure. This is supported by the observation of an increased number of circulating CD40+/CD14+ monocytes and CD40L+/CD4+/CD3+ lymphocytes in MDS patients. We have also shown that CD40-ligation induces a significantly higher TNF-α production by monocytes from patients compared to healthy volunteers. Finally, we have preliminary evidence that blocking the CD40-receptor can increase colony formation in vitro. These results mark a possible new target to treat cytopenias in MDS.

0462

IMMUNOPHENOTYPIC ANALYSIS OF CD34+ CELL SUBSETS IN BONE MARROW SAMPLES FROM MYELODYSPLASTIC SYNDROME PATIENTS

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Introduction. Current investigations regarding the presence of phenotypic aberrations among CD34+ cells in patients with myelodysplastic syndrome (MDS) have provided limited information about their fre-quency and subtypes due to the use of limited monoclonal antibody panels and/or a evaluation of insufficient amounts of CD34+ bone mar-row (BM) cells. Objectif. Our aim was to phenotypically characterize the myeloid and lymphoid CD34+ BM cell compartments in patients with MDS using a large panel of monoclonal antibodies in order to identify phenotypic alterations that may be useful for the diagnosis and classification of the disease. Material and Methods. Over 12 BM samples corresponding to 11 normal BM (NBM) and 52 BM from patients with MDS (including 19 low risk MDS patients (LR-MDS) and 33 high risk MDS cases (HR-MDS). CD34+ cells were identified and sub-b
TLR-4 (while MDS-CD34+ cells showed statistically significant higher levels of 4 in both the progenitor and differentiated cells. TNFα treatment resulted in a 70.0±2.0% increase of the TLR-4 and 20-fold increase of ICAM.1 expression. Apoptosis increased after TNFα treatment or LPS-stimulation in both BMMC and THP-1 cells, whereas an 80% reduction was observed at the mRNA level. TNFα and anti-TNFα modulations revealed that both the constitutive and the LPS-induced TLR-4 expression is TNFα dependent. The majority (73%) of the apoptotic MDS cells are TLR-4+. TLR-4 triggering leads to a strong induction of apoptosis that only to a percentage is TNRα mediated. Concluding, MDS patients over-express TNRα mediated functional TLR-4, which is implicated in both TNF-dependent and independent apoptosis.

**FUNCTIONAL TLR-4 IN MDS BONE MARROW CELLS: INVOLVEMENT OF TNFα MEDIATED TLR-4 EXPRESSION IN INCREASED APOPTOSIS**

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**Background.** In Myelodysplastic syndromes (MDS), TNFα over-expression is implicated in abnormal gene expression resulting in ineffective hematopoiesis and increased apoptosis through a Fas-mediated apoptotic pathway. Toll-like receptors (TLRs) are members of a conserved family of type I transmembrane receptors characterised by an intracellular signalling domain homologue to the IL-1R. TLR expression is induced, by TNFα and, as shown by recent studies, over-expression of TLR-4 leads to increased apoptosis through a Fas-associated pathway. Aims. In view of the excessive production of TNFα in MDS and its potential to induce TLR expression as well as the TLR-mediated apoptotic pathway, we reasoned that measurement of TLR expression levels and their functional ability in MDS cells, could provide insight to the MDS elevated apoptosis. Furthermore we examined whether anti-TNFα or anti-TLR-4 treatment affects TLR-4 expression and decreased CD33 expression in NP. In addition, in LR-MDS decreased expression of CD45 in the LP (57%), as well as decreased numbers of NP expressing CD33 (42%) and increased numbers of CD7+ NP (87% of cases), were detected. In turn, HR-MDS patients, but not LR-MDS cases, showed increased numbers of IP (38% of the cases) and low percentages of NP (56% of the cases), decreased expression of cyMPO in NP (41% of the cases) and of CD33 in both the NP (42% of the cases) and IP (56% of the cases) together with increased expression of CD117 and CD18 in both the LP (67% and 29%, respectively) and NP (48% and 37% of the patients, respectively); in addition, decreased expression of nGf and CD68 (neutrophils) and CD56 (natural killer cells), was observed. Moreover, using the CD65, CD64, CD7 and CD123, were also detected in these patients group. Conclusion. Our results show the presence of multiple phenotypic alterations in CD34+ BM myeloid and lymphoid precursors in patients with MDS, these abnormalities being more pronounced in HR-MDS as compared to LR cases.

**ABNORMALITIES OF BONE MARROW MESENCHYMAL STEM CELLS IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES**

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**Background.** Patients with Myelodysplastic Syndromes (MDS) display frequently abnormalities of the bone marrow (BM) microenvironmental cells in terms of increased production of inflammatory cytokines and defective support of haemopoiesis. Whether, however, there is a primary defect at the mesenchymal stem cell (MSC) level in these patients remains unknown. Aims. To study the reserves, the functional characteristics and the differentiation potential of BM MSCs in patients with MDS. Methods. Fifteen patients with MDS (4 with Refractory Anaemia (RA) and 11 with RA with excess of blasts (RAB)) and 22 age- and sex-matched healthy controls were studied after informed consent. The BM mononuclear cells (BMMC) and THP-1 cells were cultured in the MSCs/105 BMMC in the controls; however, patient MSCs displayed impaired CFU-F potential time-course (p=0.423) and normal immunophenotypic characteristics of BM MSCs. The chondrogenic, osteogenic and adipogenic potential of patient MSCs did not differ from the respective of the controls as was evaluated by the collagen II and aggrecan, the ALP and CBFα1 expression by RT-PCR, and chondrogenic (Masson and Alcian blue stain and Collagen II and Aggreccan expression by RT-PCR) potential after induction of differentiation in appropriate media. The frequency of MSCs in the BMMC fraction was evaluated by means of a limiting dilution assay (LDA) based on the Poisson probability. The functional characteristics of MSCs were studied by evaluating (a) their clonogenic potential using a standard colony forming unit-fibroblast (CFU-F) assay and enumerating the CFU-Fs/100MSCs plated through passages (F), (b) their proliferative potential-time course by using the MTT assay and evaluating the cell doubling time (2^n=cells counted/cells plated) in each passage. Results. MDS patients displayed normal number (18.32±13.56 MSCs/106 BMMCs in the patients versus 23.78±16.49 MSCs/106 BMMCs in the controls; p=0.423) and normal immunophenotypic characteristics of BM MSCs. The chondrogenic, osteogenic and adipogenic potential of patient MSCs did not differ from the respective of the controls as was evaluated by the collagen II and aggrecan, the ALP and CBFα1, and the alp2 and PPARγ mRNA expression, respectively, by means of a semi-quantitative RT-PCR. Compared to healthy controls, however, patient MSCs displayed impaired CFU-F potential-time course (p<0.001; P1-P7). Summary-Conclusions. Patients with MDS display normal number and differentiation potential of BM MSCs. The clonogenic and proliferative potential of patient MSCs, however, is defective compared to the respective of the healthy controls. Analysis of the production of growth factors and inhibitors at protein level, evaluation of the telomeric length as well as cytogenetic analysis of patient MSCs is currently under investigation to elucidate further the pathophysiology of the observed MSC abnormalities in MDS patients.
SIMULTEOUS SESSIONS

Chronic myeloid leukemia

0465
A RANDOMIZED STUDY OF DASATINIB VERSUS ESCALATED DOSE OF IMATINIB IN PATIENTS WITH CHRONIC PHASE CHRONIC MYELOID LEUKEMIA RESISTANT TO IMATINIB RESULTS OF CAL100017 START-R STUDY

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Background. Patients (pts) who are resistant to IM have few therapeutic options. Escalated dose of IM (800 mg/day) appears to retain some activity. Dasatinib (D) (BMS-554825) is a novel, oral, multi-targeted kinase inhibitor of BCR-ABL and SRC with activity against 18/19 BCR-ABL mutants. Aim. To demonstrate the activity of Dasatinib in pts with CP-CML, who are resistant to conventional doses of IM. Methods. START-R is a multicenter randomized (2:1 ratio) trial of D 70 mg twice daily (BID) and IM 500 mg/day in pts with CP-CML resistant to prior IM 400 to 600 mg/day. Cross-over was allowed for lack of response or intolerance (grade 3-4 non hematologic toxicity). Dose escalation to 90 mg BID was allowed for inadequate response at 12 wks, and dose reduction to 50 or 40 mg BID for drug toxicity. Dose reduction to 600 mg/day was allowed for IM. Evaluations consisted of weekly blood counts for the first 12 wks, bone marrow and cytogenetics every 3 months, molecular monitoring of BCR-ABL transcript levels by real-time quantitative polymerase chain reaction (RT-PCR) every 4 wks for the first 12 wks, and then every 12 wks, and mutation status at baseline and end of treatment. Results. From February 2005 to November 2005, a total of 150 pts were randomized (101 to D and 49 to IM). There were 75 (50%) males; median age was 51 yrs (range 24-85). Median time from diagnosis was 59 months and 34% had IM resistant mutations. Prior therapy included interferon in 107 (71%) pts, chemotherapy in 57 (38%) and stem cell transplant in 9 (6%). All pts received prior IM; 96 (64%) had 600 mg/day, 60 (40%) were treated >3 years and 42 (28%) achieved major cytogenetic response (MCyR) at 12 months (n=436), an estimated 96% were free of progression ≥ 3 log reduction of BCR-ABL transcript levels from the standardized baseline, time to progression - defined as loss of CHR/MCyR, evolution to accelerated phase/blast crisis (AP/BC), or death due to any cause during treatment, and overall survival. Results. With a median follow-up of 54 months, 72% of the 553 randomized pts remain on initial IM treatment (5% of pts discontinued due to adverse events, 9.5% due to unsatisfactory therapeutic effect and 11% due to other reasons; another 2.5% crossed over to IFN+Ara-C). Overall, the cumulative best response rates of CHR, MCyR and CCyR are 97%, 88% and 82%, respectively. The overall estimated survival was 90% (93% when censored at bone marrow transplant). An estimated 84% of pts have not progressed on centers and 93% of pts were free from progression to AP/BC. The annual rate of progression to AP/BC of <1% in the fourth year was lower than each of the first three years (1.5, 2.8, 1.6% respectively). Of the pts with MCyR at 12 months (n=456), an estimated 96% were free of progression to AP/BC at 54 months whereas it was only 81% for the 73 pts who did not achieve a MCyR at 12 months (p<0.001). No patient with a MMR at 12 months progressed to AP/BC within 54 months. Conclusions. This analysis confirms the high rates and durability of responses to IM. Encouragingly, the rate of progression in the fourth year was lower than in each of the preceding three years. Results further demonstrate the beneficial effect of cytogenetic and molecular responses on long-term outcomes.

0466
LONG-TERM BENEFITS OF IMATINIB FOR PATIENTS NEWLY DIAGNOSED WITH CHRONIC MYELOGENOUS LEUKEMIA IN CHRONIC PHASE: THE 5-YEAR UPDATE FROM THE IRIS STUDY

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Background. IM was proven to be superior to IFN+Ara-C for newly diagnosed patients (pts) with CML-CP (O’Brien et al., NEJM 2003). 1106 pts were randomized between June 2000 and Jan 2001 to either IM 400 mg or IFN+Ara-C with 553 pts to each treatment. This abstract is based on data collected up to 54 months after last patient had been recruited on IM. 60 months (5-year) data will be available for presentation. Methods. Evaluations included complete hematologic response (CHR), complete partial cytogenetic response (CCyR/PCyR - defined as 0% / 1-35% Ph+ metaphases respectively), major cytogenetic response (MCyR=CCyR+PCyR), major molecular response (MMR) defined as ≥ 3 log reduction of BCR-ABL transcript levels from the standardized baseline, time to progression - defined as loss of CHR/MCyR, evolution to accelerated phase/blast crisis (AP/BC), or death due to any cause during treatment, and overall survival. Results. With a median follow-up of 54 months, 72% of the 553 randomized pts remain on initial IM treatment (5% of pts discontinued due to adverse events, 9.5% due to unsatisfactory therapeutic effect and 11% due to other reasons; another 2.5% crossed over to IFN+Ara-C). Overall, the cumulative best response rates of CHR, MCyR and CCyR are 97%, 88% and 82%, respectively. The overall estimated survival was 90% (93% when censored at bone marrow transplant). An estimated 84% of pts have not progressed on centers and 93% of pts were free from progression to AP/BC. The annual rate of progression to AP/BC of <1% in the fourth year was lower than each of the first three years (1.5, 2.8, 1.6% respectively). Of the pts with MCyR at 12 months (n=456), an estimated 96% were free of progression to AP/BC at 54 months whereas it was only 81% for the 73 pts who did not achieve a MCyR at 12 months (p<0.001). No patient with a MMR at 12 months progressed to AP/BC within 54 months. Conclusions. This analysis confirms the high rates and durability of responses to IM. Encouragingly, the rate of progression in the fourth year was lower than in each of the preceding three years. Results further demonstrate the beneficial effect of cytogenetic and molecular responses on long-term outcomes.

Figure 1. Progression-free survival and survival without AP/BC on first-line imatinib.

0467
A PHASE II STUDY OF DASATINIB IN PATIENTS WITH CHRONIC PHASE CHRONIC MYELOGENOUS LEUKEMIA WHO ARE RESISTANT OR INTOLERANT TO IMATINIB: RESULTS OF THE CAL100013 START-C STUDY

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Background. Dasatinib (BMS-554825) is a novel, oral, multi-targeted kinase inhibitor of BCR-ABL and SRC kinases with preclinical and clinical activity against imatinib resistant BCR-ABL mutations. Aims. To demonstrate the activity of dasatinib in patients (pts) with CP-CML who are resistant to (primary or acquired resistance, or detection of BCR-ABL mutations highly associated with imatinib resistance) or intolerant (grade 3-4 non hematologic or persistant hematologic toxicity) of imatinib. Methods. START-C is an open-label Phase II study of dasatinib in imatinib-resistant (IM-R) or intolerant (IM-I) pts with CP-CML. Between February-August 2005, 387 pts were recruited at 75 centers worldwide. Dasatinib was given at 70 mg twice daily (BID) with dose escalation to 90 mg BID in pts lacking response, and dose reductions to 50 and 40 mg BID for intolerance. Evaluations were weekly blood counts
for the first 12 weeks, bone marrow cytology and cytogenetics every 3 months; and molecular monitoring of BCR-ABL transcript levels by real-time quantitative polymerase chain reaction (RT-PCR) every 4 weeks for the first 12 weeks, and then every 12 weeks while on study. The primary endpoint was major cytogenetic response (MCyR) rate. Results. Of the 387 pts, 271 were IM-R and 116 were IM-I pts. Median age was 58 yrs (range 8-71 yrs). Median time from diagnosis of CML was 61 months. Prior treatment included interferon in 252 (65%) and stem cell transplant in 38 (9.8%). All patients received prior IM; doses >600 mg/day in 214 (55%), >3 years in 207 cases (53%); 141 pts (36%) achieved MCyR on prior IM. Efficacy and safety data are currently available from 186 pts (127 IM-R, 59 IM-I) accrued prior to May 12, 2005. With 26 months of follow up, 168 (90%) pts had a complete hematologic response (CHR), and 83 (45%) pts had a MCyR; 40 (31%) of IM-R pts, and 43 (73%) of IM-I pts. Rate of MCyR was 37% among the 65 pts with BCR-ABL mutations. Grade 3/4 neutropenia or thrombocytopenia was reported in 83 (45%) pts and 65 (46%) pts with onset after 4-8 weeks of therapy in most pts. Dose interruptions occurred in 146 (78%), and dose reductions in 96 (52%) pts with an average daily dose of 108 (range 19-169) mg. Non-hematologic toxicity consisted mainly of Grade 1/2 diarrhea, headache, superficial edema, and pleural effusion, with 52% Grade 3/4. There was no cross-tolerance between dasatinib and IM. Conclusions. Dasatinib demonstrated substantial hematologic and cytogenetic activity in IM-R and IM-I pts with CP-CML. An updated analysis of 387 pts with 6 months of follow up, in addition to the molecular response analysis, will be presented.

0469

PREDICTIVE VALUE OF BCR-ABL TRANSCRIPT LEVELS AFTER 12 MONTHS OF IMATINIB MONOTHERAPY FOR THE OUTCOME OF CML PATIENTS AFTER 5 YEARS


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Background. Despite most chronic myelogenous leukemia (CML) patients (pts) treated with imatinib achieve a complete cytogenetic remission (CCR), about 4% of pts per year will relapse. The achievement of an at least 3 log reduction of BCR-ABL transcript levels compared with a predefined baseline has been associated with a favorable long term outcome. Aims. We sought to establish a relationship between 12 month quantitative PCR data and relapse free survival based on the determination of ratios BCR-ABL/ABL. Methods. Serial peripheral blood (n=574) and bone marrow (n=685) samples from 68 pts randomized for first line imatinib therapy within the international IRIS trial have been investigated employing qualitative and quantitative RT-PCR and conventional cytogenetics. Degree of molecular response was classified retrospectively using a direct comparison of the log reduction terminology and the ratio BCR-ABL/ABL. Results. Ratios of 0.01%, 0.12%, and 1.4% represent a 4, 5, and 2-log-reduction, respectively. Results. After 12 months of imatinib therapy, ratios <0.01% were achieved in 4 pts (cohort 1, 6%), ratios of 0.01-0.12% in 22 cases (cohort 2, 32%); >0.12-1.4% in 27 pts (cohort 3, 40%), and >1.4% in 15 pts (cohort 4, 22%). Overall median observation time was 57 mo and not different between the 4 cohorts. The most recent analysis showed CCR in 4/4 pts in cohort 1 (100%), 21/22 pts in cohort 2 (95%), 24/27 pts (89%) in cohort 3, and 7/15 pts in cohort 4 (47%, p=0.0006). Most recent Q-PCR values differ significantly between cohorts (cohort 1 0.001%, cohort 2 0.006%, cohort 3 0.037%, cohort 4 1.1%, p=0.0002). The same applies to overall best Q-PCR results (cohort 1 0.006%, cohort 2 0.00095%, cohort 3 0.026%, cohort 4 1.1%, p<0.0001). In 10/26 (38%) pts of cohorts 1+2 BCR-ABL was not detectable by a sensitive nested PCR at the most recent analysis whereas none of 42 pts demonstrated undetectable BCR-ABL in cohorts 3 and 4 (<0.0001). Five pts have relapsed with reappearance of Ph+ metaphases after a median of 6 mo (range 3-9) post first CCR. These pts belonged to cohorts 2 (n=1), 3 (n=2), and 4 (n=2) after 12 mo of therapy. There was a trend towards higher ratios BCR-ABL/ABL at mo 12, respectively. Conclusions. BCR-ABL transcript levels after 12 mo of imatinib therapy are predictive for long term cytogenetic and molecular response. Overall rate of CCR parallels the degree of early molecular response. A ratio BCR-ABL/ABL <0.12% is predictive for a better chance to achieve CCR accompanied by a >95% probability of CCR as well as a 38% chance of becoming nested PCR negative after 5 years. The in vivo data confirm the equivalence of the definition of a major molecular response as a 3-log reduction compared to a predefined baseline vs the ratio BCR-ABL/ABL of 0.12%.
Acute myeloid leukemia

0470
DOSE-DEPENDENT COMPETITION BETWEEN NPM LEUKEMIC MUTANTS AND ARF PROTEIN FOR SUBCELLULAR DISTRIBUTION: A NUCLEAR TUG-OF-WAR

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Acute myeloid leukemia (AML) is frequently targeted by mutations at exon-12 of the nucleophosmin (NPM) gene (Falini et al., NEJM, 352:254, 2005), which 1) disrupt either tryptophan 290 or tryptophans 288 and 290, constituting the nucleolar localization signal (NoLS); and 2) create a new carboxy-terminal Nuclear Export Signal (NES) motif, with 6 variations observed to date. Both phenomena contribute to a dramatic NPM accumulation in leukemic cell cytoplasm (NPMc+ AML). In NPM mutants, the new NES motif non-randomly correlates with NoLS disruption at the C-terminus. The most common NES motif (LxxxLxxVxL) always associates with mutations of tryptophans(W) 288 and 290, e.g. mutant A; a NES variant sequence, such as LxxxLxxVxL, always associates with W288 retention in rare NPM mutants, e.g. mutant E (Falini et al., Blood, pub-ahead, February 2, 2006). These findings suggest diverse sequences of mutant NES motif function differently. Mutated NPM dislocates Arf from nucleoli, shortens its half-life and blunts its function (Dun Besten W et al., Cell Cycle, 4:1595, 2006). In different NPM mutants this study addressed: i) the role of variations in NES motifs; and ii) interactions with Arf. i) Role of different NES motifs in altered NPM nucleo-cytoplasmic traffic: Arf-negative NIH-3T3 cells were transfected with eGFP-tagged NPM mutant A in which W288 had been artificially inserted by site-directed mutagenesis (eGFP-NPMmA_C288W). Unlike mutant A, which is unaffected by the presence of W288, this protein displayed greatly reduced cytoplasmic export. Additionally, in NPM mutant E, replacing the LxxxLxxVxL NES sequence with LxxxLxxVxL partially relocated mutant E to the nucleus. These results demonstrate efficiency differences between NES: LxxxLxxVxL is weaker than LxxxLxxVxL. The former is strong enough to export the NPM mutants only if both tryptophans are mutated whilst LxxxLxxVxL is needed if W288 is retained. ii) Interaction of NPM mutants with Arf: We investigated how changes at the NPM mutant C-terminus influence NPM-Arf binding, and NPM and Arf subcellular distribution. Arf-negative NIH-3T3 cells were co-transfected with DsRed-tagged Arf (DsRed-monomer-Arf) and eGFP-tagged NPM mutants. Arf partially relocated NPM mutants A and E from cytoplasm to nucleus in a dose-related manner. In turn, NPM mutants partially relocated Arf from the nucleolus to nucleoplasms and cytoplasm. These results demonstrate a reciprocal interaction between Arf and NPM mutants. Moreover lower doses of Arf completely relocated artificial mutants eGFP-NPMmA_C288W and eGFP-NPMmE_LVVL to the nucleus, suggesting these artificial mutants have a stronger affinity for Arf than NPM mutants A and E. Co-immunoprecipitation studies showed mutants A and E bind less Arf than wild-type NPM or eGFP-NPMmA_C288W and eGFP-NPMmE_LVVL. Conclusions: The non-random correlation between NPM NoLS disruption and NES sequence variants is feasibly explained by need for 1) efficient cytoplasmic accumulation of mutated NPM, and 2) less efficient binding of mutant NPM to Arf as compared to wild-type NPM. Both mechanisms may contribute to Arf dislocation/degradation, thus having the same functional consequences as NPM silencing. These findings may be relevant to the pathogenesis of NPMc+ AML.
0472

MICROARRAY-BASED CHARACTERIZATION OF AML WITH COMPLEX KARYOTYPES DISCLOSE NOVEL GENOMIC IMBALANCES HARBORING NEW CANIDATE GENES


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Approximately 10 to 15% of acute myeloid leukemia (AML) cases exhibit complex karyotypes, i.e., three or more chromosome abnormalities without presence of a specific fusion transcript. To identify novel genomic regions of interest in this AML subgroup we applied comparative genomic hybridization to microarrays (array-CGH) allowing high-resolution genome-wide screening of genomic imbalances. Therefore, we designed a 2.8-kb microarray consisting of 2799 different BAC- or PAC-vectors with an average resolution of approximately 2 Mb. Using this microarray platform, 83 AML cases with complex karyotypes were analyzed. Genomic losses were found more frequently than gains; the most frequent losses were deletions of 5q (71%), 17p (55%), 7q (48%); followed by deletions of 18q (30%), 16q (28%), 3p and 12q (20% each), 12p (18%), 20q (17%), and 11q (12%). The most frequent genomic gains were trisomies of 11q (59%) and 8q (51%); followed by trisomies of 1p (22%), 7q (20%), 9p (14%), 22q (13%), 13q (12%), and 6p (10%). In part, some critical segments were delineated to genomic fragments of 0.8 to a few megabase pairs in size. Furthermore, 47 high-level DNA amplifications in 19 different regions were identified; amplifications occurring in at least two cases mapped to (candidate genes in the ampiclon) 11q23.3-q24.1 (n=10; ETS, FLI1); 11q23.3 (n=2; MLL, DDXX6); 21q22 (n=5; CROP, FGF6, CCND2); 8q24 (n=4; C8FW, MYC); 9p24 (n=2; AK2); 12p13 (n=2; FGFR, CCND2); and 20q11 (n=2; ID1, BCL2L1). For better characterization of the ampiclon, we applied array-CGH using a 6.0-kb microarray with an average resolution of approximately 1 Mb revealing highly complex ampiclon structures. Furthermore, in a subset of cases we profiled global gene expression by detecting a gene dosage effect with significant lower/higher average gene expression levels across the genes located in the lost/gained regions as compared to unaltered cases. Additionally, parallel analysis displayed overexpressed candidate genes in critical amplified region, e.g., C8FW and MYC in 8q24 as well as FLI3 and CDX2 in 13q12. In conclusion, using high-resolution genome-wide screening tools such as array-CGH allows to unravel the enormous genetic diversity of AML cases with complex karyotypes, and correlation with global gene expression studies facilitates the delineation of disease-related candidate genes located in the critical regions.

0473

GENE EXPRESSION BASED CHARACTERIZATION OF NPM1-MUTATED/FLT3 ITD-NEGATIVE ACUTE MYELOID LEUKEMIA WITH NORMAL KARYOTYPE


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Background. Acute myeloid leukemia (AML) with normal karyotype encompasses a large number of molecularly distinct variants. While the presence of internal tandem duplications (ITDs) of the FLT3 (tess-related tyrosine kinase 3) gene is associated with poor outcome, recently mutations of the NPM1 (nucleophosmin) gene have been shown to be prognostically favorable. However, this effect is mainly attributed to the NPM1-mutated/FLT3 ITD-negative AML cases. While NPM1-mutated cases characterized by a distinct gene expression pattern, it remains unclear whether NPM1-mutated/FLT3 ITD-negative cases also display a characteristic signature, which might provide additional insights into the molecular basis for the good clinical outcome. Aims. Having demonstrated the presence of a signature correlated with NPM1-mutational status, we sought to define a molecular profile for AML cases with NPM1-mutated/FLT3 ITD-ITD-negative normal karyotype disease. Methods. Towards this goal, we have profiled gene expression of 138 samples of adult AML patients with normal karyotype using DNA microarray technology. All samples analyzed were derived from AML patients entered within the randomized multicenter treatment trial HD-98A of the German-Austrian AML Study Group (AMLG). Result. Based on our results, we were able to identify a 116-genes comprising expression pattern correlated with NPM1-mutated and FLT3 ITD-ITD-negative AML cases. In accordance with previous findings in NPM1-mutated cases (Alcalay et al. 2005, Verhaak et al. 2005), the NPM1-mutated/FLT3 ITD-negative pattern was also in part characterized by a prominent HOX gene cluster, which clearly separated the NPM1-wildtype from the NPM1-mutated cases. Similarly, the expression levels of BAAALC and MNI showed a correlation with the NPM1 mutational status, with NPM1-unmutated cases displaying higher expression in our data set. However, as expected the newly defined signature also defined a NPM1-mutated group that did not contain many FLT3 ITD-positive samples. This group was characterized by several interesting genes including for example TLE1, which encodes for a Groucho/TLE family protein. Groucho/TLE family proteins are transcriptional co-repressors, which mediate repression essential in embryonic development and are involved in regulation of Wnt signaling in adult tissue. Moreover, we identified several other genes of potential pathogenic relevance which also have been previously shown to be predictive in normal karyotype AML. Conclusions. Our findings support a distinct molecular mechanism associated with the favorable outcome of NPM1-mutated/FLT3 ITD-ITD-negative AML cases. Furthermore, the reported signature might contribute to improved risk stratification and clinical management of AML patients with normal karyotype disease.

0474

EFFECT OF MDR1 SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS) C1236T, G2677T AND C3435T ON MDR1 FUNCTION AND EXPRESSION IN LEUKEMIC BLASTS, AND ON TREATMENT OUTCOME IN ELDERLY PATIENTS WITH AML


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Background. The classical multidrug resistance (MDR) gene MDR1 (ABCB1) encodes for the drug efflux pump P-glycoprotein (P-gp). MDR1 expression is an adverse prognostic factor for treatment outcome in acute myeloid leukemia (AML), and is more frequently observed in older patients. Single nucleotide polymorphisms (SNPs) of the MDR1 gene, C1236T, G2677T and C3435T, have been associated with altered drug metabolism and treatment outcome. Aims. We prospectively determined these SNPs in a cohort of patients with AML of 60 years and older, and evaluated their relevance for MDR1 function and expression, MDR1 mRNA expression and clinical outcome. Methods. We have analyzed purified bone marrow derived leukemic blasts of 150 patients treated within the multtcenter, randomized phase 3 trial HOVON 51 AML (Novartis PFC C 802-E-00) (Van der Holt et al. Blood. 2005;106:2646-2654). In that trial, 119 eligible Caucasian patients aged 60 years and older with previously untreated de novo and secondary AML (FAB classification M0-M2 and M4-M7) were randomized to receive standard induction chemotherapy with or without the MDR1 inhibitor PSC-833 (Valspodar®, AMDayra, Novartis Pharmaceuticals, Basle, Switzerland). The 150 patients genotyped patients were selected for MDR1 analysis based on availability of blast samples in our cell bank. The significance of the allelic MDR1 variants of C1236T, G2677T and C3435T was evaluated with respect to P-gp expression and function in leukemic blasts, and MDR1 mRNA expression levels. The relationship between each of these genetic polymorphisms of MDR1 with clinical outcome, i.e. complete response (CR) rate, event-free survival (EFS), disease free survival (DFS) and overall survival (OS) was also assessed.

Figure 1. OS of elderly AML patients, by genotype. (A) C1236T. (B) G2677T. (C) C3435T. (D) Patients with the same variant for the 3 SNPs.
(p<0.001), contrary to other published results. Each combination of two SNPs was in linkage disequilibrium (p<0.001), which confirms results reported by Illmer et al (Cancer Res. 2002;62:955-4962). Patient baseline characteristics were not significantly different between wild-type, heterozygous or homozygous mutant patients, neither for the 3 genetic polymorphisms, nor for the patients with the same allelic variant of all 3 SNPs. P-gp efflux and expression data in purified AML blasts and in the CD34-positive subpopulation, as well as the MDR1 mRNA expression levels of MDR1 patients did not vary significantly among any of the allelic variants of MDR1. All functional and expression data were highly correlated (p<0.001). The median follow up of 24 patients still alive was 57 months (range, 8-81). No statistically significant differences in CR rate and survival endpoints were observed between the allelic subgroups (Figure 1), neither unadjusted nor adjusted for treatment arm, nor was there any apparent interaction between the allelic variants of each SNP and treatment arm with respect to outcome. Summary/Conclusions. In AML patients aged 60+, allelic MDR1 variations of C1236T, G2677T or C3435T are not associated with altered MDR1 function, nor with MDR1 expression at the transcriptional or translational level in leukemic blasts, and they do not significantly impact on clinical prognosis, suggesting that they do not exert a major impact on drug resistance in elderly patients with AML.

### Stem cell biology

**0475**

**THE MOLECULAR SIGNATURE OF PURIFIED CANCER STEM CELLS REVEALS A STEM CELL ORIGIN OF 5Q- SYNDROME**

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Background. Although it has been postulated that leukemic and other cancer stem cells frequently may originate in the corresponding rare multipotent stem cell population, conclusive evidence for such a model has only been obtained for Philadelphia chromosome-positive chronic myeloid leukemia. Aim. To identify the origin of cancer stem cells by applying global gene expression profiling and to uncover MDS stem cell specific gene expressions. Methods. Fluorescence activated cell sorting (FACS) for enrichment of candidate MDS 5q- syndrome stem cells, Fluorescence in situ hybridization (FISH) to show clonal (5q-) involvement, long-term culture initiating-cell (LTC-IC) assay to show stem cell function and oligonucleotide microarrays to evaluate the global gene expression profile of candidate MDS stem cells. Results. Global gene expression profiles of candidate MDS 5q- stem cells (CD34+CD38-Thy-1-) and normal stem cells (CD34+CD38-Thy-1+) are more similar than candidate MDS stem cells and normal progenitors (CD34+CD38-Thy-1-) are. However, BMI1 and CEBPα as up-regulated in MDS stem cells from most patients. Furthermore, these differences are specific for MDS stem cells since CEBPα is down-regulated and BMI1 is unaffected in MDS progenitor cells. Conclusions. Global gene expression profiling supports that MDS 5q- syndrome originates in normal stem cells. BMI1, a critical regulator of self-renewal, is up-regulated in MDS stem cells, as is the myeloid transcription factor CEBPα. These changes are specific for MDS stem cells and could potentially be involved in defining the MDS 5q- syndrome stem cell clonal advantage and defective differentiation process. We have demonstrated the importance of identifying the specific cancer stem cell population to uncover potential gene expression changes contributing to unique cancer stem cell properties.

**0476**

**PROTEIN KINASE B: A MOLECULAR SWITCH IN REGULATION OF LINEAGE CHOICE DECISIONS DURING MYELOPOIESIS**

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Introduction. Hematopoiesis is a highly regulated process resulting in the formation of all blood lineages. The specific signal transduction pathways involved in lineage choices during hematopoiesis remain largely unsolved. The PI3K/PKB pathway has been reported to play a critical role in proliferation and survival of cells, however, a role in regulating hematopoiesis is largely unknown. Aim. The aim of this project is to investigate whether the PI3K signaling module plays a role in regulation of myelopoiesis. Methods. Human umbilical cord blood derived CD34+ cells cultured in presence of IL-5 or G-CSF resulting in eosinophil or neutrophil differentiation, respectively, were either treated with pharmacological inhibitors to block the PI3K signaling pathway or retrovirally transduced to ectopically express constitutively active PKB, a downstream target of PKB. Results. Inhibition of PKB blocked progenitor proliferation without affecting cell survival. Interestingly, inhibition of PKB abrogated neutrophil differentiation, but conversely, dramatically enhanced eosinophil maturation. Retroviral transduction of CD34+ cells with constitutively active PKB (myrPKB) resulted in enhanced neutrophil differentiation and monocyte development, whereas eosinophil differentiation was blocked. In contrast, dominant-negative PKB (PKBcaax) induced eosinophil differentiation and inhibited neutrophil maturation. Transplantation of β2-microglobulin (−/−) NOD/SCID mice with CD34+
CONSTITUTIVE EXPRESSION OF THE ‘LYMPHOID ENHANCER FACTOR 1’ (LEF-1) PERTURBS HAEmatopoietic DEVELOPMENT AND INDUCES LEUKEMIA IN A SUBSET OF TRANSPLANTED MICE

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Background. Lef-1 is a key transcription factor of the Wnt/β-catenin signalling pathway and is crucially linked to normal B- and T-cell development. To elucidate the expression pattern of Lef-1 in different haematopoietic subpopulations and to test whether constitutive expression of this transcription factor affects early haematopoietic development, we performed by using semi-quantitative RT-PCR and Real-Time PCR. A higher frequency in WT and CA compared to the empty vector control (n=4; WT: p<0.02; CA: p<0.05). At the level of the short-term repopulating stem cell, both Lef-1 constructs remarkably increased the number of spleen colonies, resulting in a 46-fold and 7-fold increase in the CFU-S frequency in WT and CA compared to the control, respectively (median 80 (WT) and 135 (CA) CFU-S/10^5 cells versus 20 CFU-S/10^5 cells, respectively; p<0.001; WT n=7, CA n=6, control n=19). To assess the impact of Lef-1 on long-term repopulating stem cells mice were transplanted with BM cells transduced either with WT Lef-1 or CA-Lef-1. In vivo, normal haematopoietic development was severely perturbed in transplanted mice. Both constructs induced a reduction of lymphoid cells as well as a dramatic increase of myeloid cells with an inversion of the lymphoid/myeloid ratio (WT: ratio 0.43, p<0.01; CA: ratio 0.10, p<0.001; vs. 1.02 in control mice). Engrafted mice succumbed to a lethal myeloproliferative syndrome and/or acute leukemia, which were readily transplanted into secondary recipients and showed indefinite IL-3 dependent cell growth in vitro. Conclusions. These data show that balanced expression of Lef-1 plays a key role in early haematopoietic development and that deregulation of this transcription factor favours the development of myeloid malignancies.

CD9 IS DIFFERENTIALLY EXPRESSED ON MURINE HEMATOPOIETIC STEM CELLS AND PROGENITOR CELLS

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CD97 is a member of the EGF-TM7 family of class II seven-span transmembrane receptors and is broadly expressed on hematopoietic cells including lymphocytes, granulocytes, and monocytes. We have recently demonstrated that CD97 is involved in IL-8-induced hematopoietic stem cell (HSC) mobilization (Blood 2003; 102:455a). To determine a possible role of HSC in this process, we studied the expression of CD97 on HSC. Murine HSC are characterized as c-Kit+PO1c. Analysis of CD97 on HSC was performed by FACS analysis. The frequency of CD97- cells on bone marrow (BM) cells was 3.5% (negative controls) compared to 10.8% of CD97+ cells from wild-type animals. These results indicate that CD97+ cells could be sequentially recloned for up to 40 days. By this time, CD97+ cells present in the BM, could be single cell level and their progeny resembled in morphology the cell lines obtained from Gata1low mice in BMMC. Conclusions. These results indicate that CD97+ cells, but not GFP, are present in the tissues of the Gata1low mice. However, in these mutants, the mast cell generation was abnormally affected by CD97, which represents the unique tri-lineage progenitor previously identified in the tissues from these mutants. Therefore, Gata1low MEP are antigenically but not functionally, equivalent to MEP from wild-type animals. These results indicate as new target for Gata1low mutation the restriction point when CD97 became committed toward ME/GPR or MF.

ALtered FOSTERING/Differentiation POTENTIAL OF COMMON MEGAKARYOCYTIC-EryTHROID PROGENITORS FROM MICE CARRYING THE HYPOMORPHIC GATA1 LOW MUTATION

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Background. Several recent evidences suggest that, in addition to its function at late stages of maturation, Gata1 also controls the proliferation/differentiation potential of hematopoietic progenitor cells. We have previously shown that the hematopoietic tissues from mice carrying the hypomorphic Gata1low mutation contain numerous (~10%) of all the cells unique tri-lineage progenitor cells, committed toward the erythroid, megakaryocytic and mastocytic lineage (Migliaccio et al. J. Exp. Med. 2003,197:281). Although predicted by the stochastic model of hematopoietic commitment, the nature of these cells is unclear because progenitors with such a biological function have not been prospectively isolated from mouse tissues as yet. Aims. To clarify the effect of the Gata1low mutation at the level of the hematopoietic progenitor cells by prospectively identifying the tri-lineage progenitors present in the tissues of these mutants. Methods. The number and function of mast cells from Gata1low mice, normal littermates (positive controls) and W/Wv mutants (negative controls) was compared in bone marrow derived mast cells. Results. Gata1low mast cells showed different phenotypes to those of normal mast cell progenitors (the common myeloid (CMP), granulocytic-monocytic (GMP), megakaryocyte-erythroid (MEP) and mast cell (MCP) progenitors in the marrow and spleen from wild type and Gata1low littermates was compared on the basis of specific antigen profiles. The biological functions of these cells was investigated in single cell cultures followed by single cell replating experiments. Results. BMMC from Gata1low mice generated different types of the common myeloid (CMP), granulocytic-monocytic (GMP), megakaryocyte-erythroid (MEP) and mast cell (MCP) progenitors in the marrow and spleen from wild type and Gata1low mice, normal littermates and Gata1low mice, respectively. In addition, FACS analysis revealed that the majority (82.6%) of CD97high (CD97HI) subset (repopulation rate 90% versus 0% in the CD97low subset). These results indicate that CD97+ cells, but not GFP, are present in the tissues of the Gata1low mice. However, in these mutants, the mast cell generation was abnormally affected by CD97, which represents the unique tri-lineage progenitor previously identified in the tissues from these mutants. Therefore, Gata1low MEP are antigenically but not functionally, equivalent to MEP from wild-type animals. These results indicate as new target for Gata1low mutation the restriction point when CD97 became committed toward ME/GPR or MF.
Dendritic cells, vaccination and cellular immunotherapy

0480
CLINICAL BENEFIT ASSOCIATED WITH IDIOTYPIC VACCINATION IN FOLLICULAR LYMPHOMA
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Background. So far, no human tumor vaccine has proved beneficial to any cancer patients. Aims. The formal demonstration of such an efficacy is currently widely sought, particularly in the setting of idiotypic vaccination for follicular lymphoma (FL). However, standard randomized trials struggle with the major flaw of experimental arms in which each and every patient ultimately undergoes a different, customized treatment. Methods. For this reason, we have instead conducted a phase II study of ELISPOT and vaccination start. The duration of this period was based on the time to recovery of normal numbers of circulating CD3+, CD4+, CD8+ responses by 5 independent methods, and minimal residual disease (MRD) by 3 independent methods. Results. Of the 25 patients who were actually vaccinated, 20 have specifically responded to the vaccine with a clinical complete response (CR) following monthly CHOP-like chemotherapy (CHT) were to receive up to 10 idiotypic vaccinations over 23-27 months, depending on the duration of the length of time between CHT completion and vaccination start. The duration of this period was based on the time to recovery of normal numbers of circulating CD3+, CD4+, CD8+ and CD19+lymphocyte subpopulations. From October 2001 to June 2004, thirty-three patients were enrolled. Eight of them ultimately were not vaccinated for a variety of reasons such as failure at producing the vaccine, early relapse or both. In the vaccinated patients, specific humoral responses were assessed by standard ELISA, specific cellular responses by 3 independent methods, and minimal residual disease (MRD) by 3 independent methods. Results. Of the 25 patients who were actually vaccinated, 20 have specifically responded to the vaccine with a humoral (13/20) and/or a cellular response (18/20), while five have not. From a clinical standpoint, the median duration of the first clinical CR of the 25 patients vaccinated was 17 months (range: 5-65); their current median follow-up is 21 months after the salvage treatment above. Among them, 19/25 had received at diagnosis a treatment that might be considered comparable in activity to the CHT used at the time of relapse. Moreover, no patient had lower stage, FLIPI score or histological grade for relapse, MRD did not specifically correlate with clinical outcome. Remarkably though, no patient who made a vaccine-induced, sustained and specific immune response relapsed during the 26-30 months of active vaccination (0/20). All responding patients with a sufficient follow-up experienced a second clinical CR longer than both their corresponding first clinical CR (17/17; p<0.0001 by both standard and adjusted-for-matching log rank test) and the median duration of a CHOP-induced second clinical CR, generally estimated in 13 months (20/20). By contrast, all 5 patients in whom the vaccine completely failed to elicit an immune response had a second clinical CR shorter than the first clinical CR, and relapsed within 6-12 months after having achieved second remission. Conclusions. Taken together, these results provide the first evidence ever of clinical benefit associated with the use of a human cancer vaccine.

0481
RHAMM/CD168-R3 PEPTIDE VACCINATION OF HLA-A2+ PATIENTS WITH ACUTE MYELOID LEUKEMIA, MYELODYSPLASTIC SYNDROME AND MULTIPLE MYELOMA ELICITS IMMUNOLOGICAL AND HEMATOLOGICAL RESPONSES
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Background. The receptor for hyaluronic acid mediated motility (RHAMM/CD168) RHAMM/CD168 is expressed in more than 80% of acute myeloid leukemia (AML) or multiple myeloma (MM). Recently, we characterized RHAMM/CD168 as a leukemia-associated antigen (LAA) eliciting both humoral and cellular immune responses in patients with hematological malignancies. Furthermore, we defined the RHAMM/CD168-derived peptide R3 (ILSLELMKL) as a CD8+ T cell epitope. R3-primed CD8+ T lymphocytes were able to lyse autologous RHAMM/CD168+ AML blasts in a MHC class I-restricted and epitope-specific manner. Aims. We therefore initiated a phase I/II R3 peptide vaccination to induce immunological and hematological responses for patients with AML, MDS or MM overexpressing RHAMM/CD168 to induce immunological and clinical responses. Methods. Patients were included with positive RHAMM/CD168 expression but with a limited tumour load. At a biweekly interval, 300 mcg RHAMM R3 peptide emulsified with the incomplete Freund’s adjuvant (day 0) and GM-CSF (100 mcg, days 1-5) was administrated four times subcutaneously. The primary aim of the study is safety and feasibility of this peptide vaccination, secondary aims the evaluation of a specific T cell immune response to RHAMM/CD168 R3 peptide and the assessment of the influence of the R3 peptide vaccination on the remission status. Since January 2005, twelve patients were enrolled in this study. Results. The first ten patients (2 AML, 4 MDS, 4 MM) have completed the course of four vaccinations and four patients have been evaluated. The only side effects observed under R3-peptide vaccination were erythema and induration of the skin at the site of injection (CTC ≥ 3). In 7/10 patients, we found in the peripheral blood a significant increase of specific CD8+ T cells (from 0.01% to 0.8%) recognizing the R3 peptide in ELISPOT analysis and seven-color flow cytometry including tetramer staining, two patients showed already initially a high number of HLA-A2/R3 tetramer+WT1tetramer-CCR7-CD27+CD28+CD45RA+ effector T cells and maintained this level of T cell response. Clinical responses have been assessed by the examination of peripheral blood and bone marrow samples before and after vaccination. Patients showed a reduction of the tumor-specific expressed antigen RHAMM/CD168 in real-time RT-PCR analysis after vaccination. 3/7 patients with myeloid disorders (1 AML, 2 MDS/RAEB1) showed a reduction of CD33+ cells in FACS analysis of the bone-marrow after four vaccinations from 10 and 7% to 1-2 and <1%, respectively. Two patients with MM showed a reduction of plasma cells in bone-marrow and a stable quantity of light chains in peripheral blood, one patient with AML showed a progressive disease. Conclusion. 70% of immunological and 40% of hematological responses were observed. RHAMM/CD168 is therefore a promising target antigen for immunotherapies in patients with hematological malignancies.

0482
TRIGGERING OF P38 MAPK BY CONDITIONAL MKK6 INDUCTION IS SUFFICIENT FOR DENDRITIC CELL MATURATION, AN EFFECT FURTHER ENHANCED BY INHIBITION OF NUCLEAR RELB
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Activation of Langerhans cells (LCs) by diverse signals involves p38 MAPK phosphorylation. Whether p38 is sufficient to trigger LC activation remains unknown. We show that conditional induction of a dominant active form of MAPK kinase 6 (d.a.MKK6), a direct upstream kinase of p38, in LCs is sufficient to induce the upregulation of co-stimulatory molecules and to enhance their T cell stimulatory capacity. These immediate effects showed no or only a minor requirement for classical NF-kB signaling. Concomitant with LC activation, d.a.MKK6 strongly induced the alternative NF-kB member RelB, whose nuclear localization marks mature DCs. Specific inhibition of nuclear RelB during MKK6-induced LC activation further enhanced their maturation state, thus suggesting a novel LC intrinsic control mechanism regulated by RelB.
THE IMMUNE RESPONSE TO BCR-ABL PEPTIDE IMMUNISATION IS VARIABLE AND TRANSIENT IN CHRONIC MYELOID LEUKAEMIA: RESULTS FROM THE EPIC STUDY

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Chronic myeloid leukaemia (CML) is characterised by the BCR-ABL oncoprotein. The peptide sequences spanning the junctional region are completely leukaemia-specific. Vaccination with these peptides could therefore elicit/augment an immune response directed to CML cells. Entry requirements to our ongoing Evaluation of Peptide Immunisation in CML (EPIC) study were all of the following: first chronic phase of CML, expression of the e14a2 (b3a2) BCR-ABL transcript, and prior treatment with imatinib at a stable dose of at least 400 mg daily for at least 8 months. Each patient received intradermally a cocktail of 5 BCR-ABL peptides: (1) a 9-mer spanning the e14a2 region, (2) this same 9-mer linked to a PADRE (a 15-mer non-natural peptide shown to activate CD4+ T cells, to which all patients are immunologically naive), and (3) a 13-mer consensus e14a2 junctional peptide linked to PADRE. These peptides were administered at either 100 (5 patients), 300 (5 patients), 600 (5 patients), or 1000 µg (3 patients) with sargramostim on 6 occasions over 2 months. Immune responses to the vaccine were monitored by IFN-γ and IL-5 ELISPOT assays on peripheral blood mononuclear cells. Currently, 18 patients are evaluable at 6 months of follow-up. At entry, no patient showed a detectable immune response to the PADRE peptide, but all 18 patients had detectable T cell responses within 3 months of commencing vaccination. These typically persisted at 5 and 6 months (i.e. 3 and 4 months after completion) of vaccination. These anti-PADRE responses were carried out by CD4+ T cells as demonstrated by flow cytometry analysis of IFN-γ-producing cells, and indicated that the vaccination protocol was capable of stimulating T cells in all 18 patients. Immune responses to BCR-ABL junctional peptides were monitored using the 9-mer sequence used in the vaccine and an 18-mer spanning the whole junctional region. In all but one case, there was no evidence of T cell responses to these peptides pre-vaccination. Upon vaccination, IFN-γ-producing cells to the 9-mer peptides were detected in 11/18 patients, and these cells were demonstrated to be CD8+ T cells by flow cytometry analysis. Moreover, CD4+ T cells specific for the 18-mer junctional peptide were detected in 14/18 patients. Interestingly, immunophenotyping indicated that these BCR-ABL-specific T cells were of a memory phenotype (CD45RO+). However, the anti-BCR-ABL responses were typically transient, disappearing by 5 months (i.e. 3 months after completing vaccination) in all but one case, in sharp contrast to the responses to PADRE. A good correlation was observed between the presence of BCR-ABL-specific T cells and a decrease in the level of BCR-ABL transcripts. These data demonstrate that peptide vaccination can elicit anti-BCR-ABL peptide responses in CD8+ and CD4+ T cells, but these are less frequent and less durable than those to the novel antigen PADRE. This suggests that BCR-ABL is either a weak antigen or that patients may be tolerised to it. Further functional characterisation (e.g. granzyme B production) of these BCR-ABL specific T cells is currently ongoing.

MINOR HISTOCOMPATIBILITY ANTIGENS ENCODED BY ATP DEPENDENT HAEMOLYTIC PROTEINase GENE


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Minor Histocompatibility antigens (mHag) play an important role in both graft versus tumor effects and graft versus host disease (GVHD) after allogeneic stem cell transplantation (SCT). From a patient with a multiple myeloma after allogeneic SCT entering complete remission after donor lymphocyte infusion (DLI) coinciding with mild GVHD, several T cell reactivities were isolated at the time of the clinical response. The most dominant T cell reactivity showed HLA-A2 restriction and strongly recognized tumor cells and activated B and T cells (PHA-blasts), whereas resting T cells were only moderately recognized. From mHag positive EBV-LCL peptides were isolated, tested for reactivity and subjected to sequence analysis. One candidate peptide reconstituted CTL reactivity. This peptide was identical to an alternatively translated protein sequence derived from the human ADIR gene. A SNP in this gene resulting in an aminoacid change in the candidate peptide was shown to be present in patient cells. Synthetic peptides of both patient and donor SNP variants were synthesized and specific recognition of the identified patient derived peptide was demonstrated. Transfection experiments with plasmids containing patient or donor ADIR gene constructs confirmed involvement of this SNP in T cell recognition. A population study revealed 100% correlation of the presence of the relevant SNP with recognition of PHA-blasts in 51Cr release assays. The SNP was present in 43 out of 76 individuals tested. We designated the epitope as LB-ADIR-1F. Tetramer analysis of patient samples that were taken after DLI showed up to 2.6% LB-ADIR-1F specific T cells coinciding with conversion to remission. It was previously shown that IFNα could upregulate ADIR gene expression, and the patient was treated with IFNα during DLI. Therefore, SNP positive MNC were cultured with IFNα prior to addition of the LB-ADIR-1F CTL. IFNα increased both susceptibility to lysis and stimulatory capacity of pretreated MNC. Quantitative PCR showed increased ADIR mRNA levels in IFNα stimulated cells thus supporting the role of IFNα in ADIR gene expression. Recognition of SNP positive mesenchymal stem cells as a representative of non hematopoietic cells was low and growth arrest further decreased recognition. Analysis of the immunological response in this patient also revealed T cell reactivities directed to the mHag LB-ECGF-1H and HA1. The sum of the percentages of these circulating mHag specific T cells at the time of the clinical response was 4.3%, approaching the total number of activated circulating CD8+ T cells in the patient as measured by HLA-DR expression suggesting that these 3 reactivities were responsible for the clinical course. Whereas expression of HA1 and ECGF is relatively restricted to hematopoietic cells, ADIR gene expression is more broad. LB-ADIR-1F specific T cells were shown to be highly cytotoxic for multiple myeloma cells and other hematological malignancies. Since only mild acute GVHD was observed which rapidly disappeared after discontinuation of the IFNα treatment and administration of corticosteroids, we hypothesize that the activation status of GVHD target tissues determines the clinical outcome of treatment with LB-ADIR-1F specific T cells in adoptive immunotherapy.
Vascular biology and granulocytes

0486
INHIBITION OF HIF-1α BY A POTENT RNA ANTAGONIST IS ASSOCIATED WITH MULTIPLE MECHANISMS OF ANTI-TUMOUR ACTIVITY


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Substantial evidence has accumulated to demonstrate that over-expression of Hif-1α is associated with tumour angiogenesis, tumour progression and poor prognosis in a broad range of cancers. It thus represents a potential point of intervention for targeted therapies for this group of cancers. The unique properties of Locked Nucleic Acid (LNA) chemistry have been used to generate a single stranded RNA antagonist to the Hif-1α mRNA (SPC2968), which exhibits increased stability, improved resistance to nucleases and much higher binding affinity to the target, than other second and third generation oligonucleotides. We demonstrate that SPC2968 potently inhibits Hif-1α expression in vitro (IC50 < 1nM) and that this is correlated with parallel inhibition of Hif-1α regulated genes in vitro under hypoxic and ischemic conditions. Hif-1α knock down in cancer cell lines was also correlated with increased induction of apoptosis and cell death. Following administration of a single dose of SPC2968 to wild type mice, liver Hif-1α mRNA levels were substantially reduced for periods up to 5 days. Hif-1α inhibition was also correlated with reduced expression of genes regulated by Hif-1α, namely MMP2 and VEGF. Ex vivo assays of endothelial tube formation and aorta ring outgrowth demonstrated that SPC2968 administration was also associated with impaired ability of endothelial cells to form capillaries and sprouts. Potent anti-tumour effects of the drug were also observed in murine xenograft models, both when tumour cells were transfected with SPC2968 prior to implantation and when pre-treated tumours were subsequently treated in the host during the study, suggesting that both initial and later phases of tumour growth can be impeded by Hif-1α down-regulation. The bio-distribution of SPC2968 following intravenous administration, as assessed by whole body autoradiography using tritium-labelled SPC2968, showed extensive tissue distribution with the LNA oligo still detectable in the kidney 21 days after the injection. Fluorescent-labelled SPC2968 distribution and cellular localisation were additionally investigated in several organs including skin, tumor, liver, kidney and bone marrow. All cell lineages tested were found to be positive for the label. Correlation between uptake of SPC2968 and Hif-1α expression was also addressed by HPLC and QPCR analysis in different tissues. Overall we provide data supporting the therapeutic use of a single-stranded, LNA-based RNA antagonist to target Hif-1α, and conclude that this important transcription factor may act at several points in tumour development.

0487
CONCURRENT MUTATIONS IN NEUTROPHIL ELASTASE AND GRANULOCYTE-COLONY-STIMULATING FACTOR RECEPTOR GENES IN A CASE OF SEVERE CONGENITAL NEUTOPENIA

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Background. Severe congenital neutropenia (SCN) is a heterogeneous disorder characterized by extremely low levels of circulating neutrophils, and a propensity for myelodysplastic syndrome and acute myeloblastic leukemia. Germline mutations in the ELA2 gene, encoding neutrophil elastase, are the cause of the disease in 65-80% of the cases. In contrast, mutations in the CSF3R gene, encoding granulocyte colony-stimulating factor receptor (G-CSF-R), are found in approximately 20% of SCN patients and are almost universally acquired. They typically lead to truncation of the intracellular domain of the receptor and result in extended signaling, particularly of STAT5, that may play a role in the predisposition of SCN patients to leukemia. However, we have previously described an SCN patient with a constitutive mutation in the G-CSF-R extracellular domain that results in hyperresponsiveness to ligand and suppressed STAT5 signaling. To further our understanding of SCN etiology through a re-examination of this patient with respect to the status of the ELA2 and CSF3R genes.

Methods. Genomic DNA and hematopoietic cell-derived cDNA were analysed for the presence of mutations in the ELA2 and CSF3R genes. Compound G-CSF-R mutants were then examined for their ability to activate STAT5.

Results. A novel germline ELA2 mutation was identified in this patient, causing a frameshift after P205 and a premature stop. In addition, two independent truncating mutations within the G-CSF-R intracellular domain, R710X and Q718X, were detected at different times in this patient. In vitro studies demonstrated that such intracellular truncations could partially restore the STAT5 response in the context of the extracellular P206H mutation. Summary/Conclusions. These data add to our understanding of the etiology of SCN adding to the evidence that ELA2 mutations are a likely primary cause. These may be exacerbated by CSF3R mutations, particularly those in the extracellular domain that affect sensitivity to G-CSF. This further would suggest the usefulness of the neutrophil environment conducive to the subsequent expansion of cells expressing truncating G-CSF-R mutations. In addition, these results further attest to the importance of STAT5 in mediating responses to G-CSF.
NEOPLASTIC CIRCULATING ENDOTHELIAL CELLS IN HEMATOLOGIC MALIGNANCIES

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Background. Several studies have shown that bone marrow-derived endothelial cells (EC) may contribute to tumor angiogenesis and that in the peripheral blood of cancer patients there is an increased amount of circulating ECs (CECs) that may participate to new vessel formation. Recent data also showed that microvascular ECs in B-cell lymphomas are in part tumor-related reflecting a novel aspect of tumor angiogenesis. All together these observations suggest that tumors can elicit the sprouting of new vessels from existing capillaries through the secretion of angiogenic factors and that, in some cases, cancer cells can also mimic the activities of ECs by participating in the formation of vascular-like networks. Aims. To clarify if, in different hematologic malignancies with known cytogenetic aberrations, CECs are tumor-derived. Methods. We studied 21 patients with different hematologic malignancies (6 MM, 2 CML, 5 AML, 1 ALL and 7 CLL). To isolated CECs, we used a dual step immunomagnetic sorting by means of CD45 and CD146 antibodies. By using immunomagnetic sorting in combination with CD45, we first eliminated all hematopoietic cells, which are CD45 positive, without affecting the EC component, which is characteristically CD45 negative. We then sorted CECs by means of CD146, an antigen expressed almost exclusively on ECs and absent on hematopoietic cells. To confirm the EC commitment, we then performed additional phenotypic studies with antibodies recognizing endothelial and neoplastic cells. FISH analysis was finally performed on sorted CECs with different commercially available probes in dual colour experiments. Results. In all experiments more than 95% of immunomagnetically sorted cells were of EC origin as demonstrated by phenotypic analyses. After immunomagnetic selection less than 0.5% of cells were CD45+ while CD14 was expressed in 0.1% of all immunomagnetically sorted CECs. More than 95% of immunomagnetically sorted CECs expressed VEGFR2, vWF, CD144 and UEA-1 lectin. Very few immunomagnetically sorted CECs expressed antigens expressed on neoplastic cells (CD138, CD38, CD35, CD19, CD5). FISH analysis showed that a significant proportion of CECs was tumor-derived because they harbored the same genetic lesion as observed in neoplastic cells. The fraction of CECs showing the cytogenetic aberration averaged 20% (range, 11-34%), 200 cells observed in each case). The majority (>85%) of CECs presented features of EPCs because they expressed CD135, a marker gradually lost during EC differentiation and absent in mature ECs. Overall, 98.0% of CECs with genetic lesions were CD135 positive. Conclusions. These findings suggest that in many hematologic malignancies CECs are in part tumor related and with EPC features. These CECs may contribute to tumor neovascularogenesis and possibly to the spreading and progression of the disease. It is possible to speculate that neoeplastic CECs may have arisen from a common hemangioblast precursor that can give rise to both neoplastic cells and ECs or alternatively through a process of dedifferentiation of a already committed cell into a cell with EPC characteristics followed by a redifferentiation into a terminally differentiated EC. Disguised plasma cells may then mimic functional CECs and contribute to tumor neovascuogenesis.

EVIDENCE FOR DISTINCT PHENOTYPIC STAGES DURING ENDOTHELIAL PROGENITOR DIFFERENTIATION

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Background. Despite the importance of endothelial progenitor cells (EPC) in tumor angiogenesis, little is known about the mechanisms that regulate their differentiation towards mature endothelium (EC). Aims. In the present project, we hypothesized the process of EPC>EC differentiation might involve distinct phenotypic/functional phases, which may be characterized by the expression of particular genes/gene products and thus may be suitable for therapeutic targeting. Methods. To define whether this is the case, we conducted cDNA- oligonucleotide microarrays (Superarray and Affymetrix) analysis of human umbilical cord blood CD34+ cells, cultured in EC differentiation medium, at different time-points after the start of culture. Results. The initial characterization (day 0) of freshly isolated CD34+KDR+CD117+ cord blood cells revealed a gene expression pattern, which included stress related genes (tnf, lfnx1, tgfβ1), those coding for matrix-specific receptors (Integrins α 1 and 5) and some involved in particular signalling pathways (Raf1, Ikrb). As the cells respond to the growth factors in the culture medium, the first stage of EC differentiation (day 5) revealed an increase in the expression of specific integrins, receptors (Fgfr2, Fth1, Tek), an up-regulation of cell cycle (Cdkn1B, Rad53, Ccnd1), and apoptosis-related genes (Bcl2l1, Tnfrsf1A, Casp8). In contrast, genes indicative of mature EC function (Il-8, thrombospondin 2, Timp1) were expressed solely at the end of the differentiation assay (days 23-26). Summary/ Conclusions. We suggest the following stages regulate endothelial differentiation from EPC: 1) adherence to a particular matrix; 2) response to specific growth factors, promoting proliferation and survival; 3) maturation and acquisition of endothelial functions. These results reveal new putative targets with therapeutic potential, and suggest that strategies aimed at blocking EPC recruitment and differentiation (to halt tumor angiogenesis), should be designed specifically against defined target genes. We are now performing RNAi studies to test the function of specific genes (integrins, chemokines) during endothelial differentiation.
Multiple Myeloma - Clinical

A PROSPECTIVE RANDOMIZED CONTROLLED TRIAL OF ORAL MELPHALAN, PREDNISONE, THALIDOMIDE VERSUS ORAL MELPHALAN, PREDNISONE IN ELDERLY NEWLY DIAGNOSED MYELOMA PATIENTS

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Background. For patients older than 65 years of age oral melphalan and prednisone (MP) has remained the treatment of choice since 1960. So far no major improvement in outcome from the original combination MP has been achieved in these elderly patients and new treatments are urgently needed. In this multicentre randomised trial we compared oral melphalan and prednisone plus thalidomide (MPT) with MP alone in 60 to 85 years old patients. Aims. The primary objective was to compare the clinical response rates and the event-free survival in the two treatment groups. Secondary end points included overall survival, prognostic factors, time to the first evidence of response and incidence of any grade 3 or higher adverse events. Methods. The trial was conducted at 54 centres in Italy. Patients with newly diagnosed multiple myeloma were randomly assigned to receive oral MP (N=129) for six four-week cycles plus thalidomide 100 mg per day continuously until any sign of relapse or progressive disease (Pharmion LTD, Windsor, UK) or MP alone (N=126). After the study was amended and enoxaparin at 40 mg per day was delivered subcutaneously during the first four cycles of therapy. Results. Patients treated in MPT arm experienced higher response rates and a longer event-free survival than patients who were not. In intention-to-treat analysis, the complete and partial response rates were 76.0% for MPT and 47.6% for MP alone (absolute difference +28.3%, 95% CI 16.5 to 39.1), and the near complete and complete response rates were 27.9% and 7.2%, respectively. The two-year event-free survival rate was 54% in patients receiving MPT and 27% in patients receiving MP. The hazard ratio (HR) for MP was 0.66 (CI 0.48 to 0.85, p<0.001). This is a 49% decrease in the risk of events in the MPT group. The three-year survival rate was 80% in patients taking MPT and 64% in patients taking MP, the HR for MPT was 0.66 (95% CI 0.38 to 1.22), p=0.19. Grade 3-4 adverse events were 48% in MPT patients and 25% in MP patients (p<0.001). In the MPT group, the most frequent grade 3-4 adverse events were haematological, thromboembolism, infections and peripheral neuropathy. The introduction of enoxaparin prophylaxis significantly reduced the incidence of thromboembolism from 20% to 3% (p=0.005). Conclusion. Oral MPT is superior to MP as first-line treatment for elderly patients with multiple myeloma. Anticoagulant prophylaxis reduced the frequency of thrombosis. Longer follow-up is needed to assess the effect on overall survival.

ORAL REVLMID PLUS MELPHALAN AND PREDNISONE FOR NEWLY DIAGNOSED MULTIPLE MYELOMA: A PHASE I-II STUDY

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Background. Lenalidomide (Revlimid®) is a novel, orally active immunomodulatory drug effective for the treatment of relapsed and refractory myeloma. Lenalidomide has shown additive effects with melphalan and corticosteroids. No data are available on the clinical use of Lenalidomide in combination with the oral melphalan and prednisone (MP). Aims. In this multicenter phase I-II study, we evaluated the safety and efficacy of different doses of Revlimid® in combination with melphalan and prednisone (R-MP). Methods. Between December 2004 and November 2005, 54 newly diagnosed symptomatic MM patients (pts) older than 65 years were enrolled. Pts were treated with 9 courses of Revlimid, (5-10 mg/day for 21 days every 4-6 weeks) plus MP (melphalan 0.18-0.25 mg/kg/day and prednisone 2 mg/kg/day for 4 days every 4-6 weeks). Four different dose levels were tested: 1. melphalan 18 mg/kg + Revlimid® 5 mg/day; 2. melphalan 0.25 mg/kg + Revlimid® 5 mg/day; 3. melphalan 0.16 mg/kg + Revlimid® 10 mg/day; 4. melphalan 0.25 mg/kg + Revlimid® 10 mg/day. Each cohort included 6 pts, with additional 15 pts enrolled at dose level 3 and 4. Dose limiting toxicity (DLT) was defined as: any grade > 3 non-hematologic toxicity; grade 4 neutropenia lasting >7 days; any other grade 4 hematologic toxicity and any treatment delay due to toxicity that occurred during the first cycle. All pts received ciprofloxacin and aspirin as prophylaxis. Results. 30 pts (median age 71, range 57-77) were evaluated after at least one R-MP course. No DLTs were observed in the first 2 dose levels. In level 3 one pt experienced DLT (grade 4 neutropenia lasting >7 days). In level 4 three pts experienced DLT (1 pt experienced neutropenic fever and grade 3 cutaneous toxicity, 1 pt had pulmonary embolism, 2 pts had a delay in the start of cycle 2 due to hematologic toxicities). After 1 cycle of R-MP, no one was in complete remission (according to the EBMAT/IBMR criteria), 16% of pts showed myeloma protein reduction of 75-99%, 35% myeloma protein reduction of 80-74%, and 49% reduction <50%, no disease progressions were observed. After 3 cycles of R-MP, complete remission was observed in 10% of pts, myeloma protein reduction of 75-99% was detected in 30%, response of 50-74% in 30% and response <50% in 30%, no disease progressions were observed. Major grade 3 or 4 adverse events consisted of hematological toxicities: neutropenia (58%), thrombocytopenia (21%) and anemia (15%). Major grade 0 or 1 non-hematological toxicities recorded were cutaneous eruption (11%), infection (5%) and febrile neutropenia (3%). Neutropenia was not observed and only one case of thromboembolic events (pulmonary thromboembolism) was recorded. Conclusion. R-MP represents a feasible and promising approach for newly diagnosed pts who are not candidates for transplant. It was well tolerated with a manageable toxicity and showed a significant response rate. An update of these data will be presented.

HAEMATOLOGICAL PROFILES WITH BORTEZOMIB OR HIGH-DOSE DEXAMETHASONE TREATMENT IN RELAPSED MULTIPLE MYELOMA: PHASE 3 APEX TRIAL

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Background. Bortezomib (VELCADE®) is a novel proteasome inhibitor that has demonstrated safety and efficacy for patients with relapsed and/or refractory multiple myeloma in phase 2 and 3 trials. Bortezomib was associated with thrombocytopenia and neutropenia in SUMMIT (NEJM 2003;348:2609) and CREST (BJH 2004;127:165) and both were transient and cyclic. Aims. This analysis characterised the haematological profiles of patients treated with bortezomib or high-dose dexamethasone in APEX, the largest phase 3 trial in patients with relapsed multiple myeloma following 1-3 prior therapies (NEJM 2005;352:2487). Methods. 669 patients with relapsed multiple myeloma were randomised to bortezomib 1.3 mg/m², d 1, 4, 8, 11 q3wk for 8 cycles, then 3 cycles on d 1, 4, 8, 11 q5wk, or dexamethasone 40 mg, d 1-4, 9-12, 17-20 q5wk for 4 cycles, then 5 cycles on d 1-4 q28d. Data on adverse events, laboratory values, and transfusion experience were collected at baseline and regularly through therapy. Results. Anaemia, neutropenia, and thrombocytopenia reported as adverse events are shown (Table). The incidence of anaemia was similar in both treatment arms. 53% of patients on bortezomib and 20% of those on dexamethasone received blood transfusions for anaemia. Patients on bortezomib experienced a steady increase in haemoglobin over time and the requirement for blood transfusions decreased over time to 0% after cycle 4. Bortezomib-associated neutropenia was also transient and cyclic, and febrile neutropenia...
nia was rare. G-CSF or GM-CSF was used at a low rate to manage neutropenia. Thrombocytopenia was cyclical, with recovery towards baseline during the rest period of each cycle. Overall, 15% of patients on bortezomib and 1% of those on dexamethasone received platelet transfusions for thrombocytopenia. Preclinical study of the effect of bortezomib on megakaryocytes indicates a shorter recovery time than with cytotoxic marrow injury, an absence of a lethal cytotoxic effect, and no cumulative or persistent thrombocytopenia. The number of patients requiring platelet and blood transfusions peaked within the first 2 cycles in both treatment arms. Although the number of platelet transfusions was higher with bortezomib, the number of significant bleeding events (including any grade 3/4, any with an intensity reported as serious, and cerebral haemorrhage regardless of intensity and seriousness) was similar in the 2 arms. No difference was observed in response rate or duration of response in patients who received platelet transfusions compared with patients who did not need platelet transfusion. Median duration of therapy for platelet-transfused patients was 5.8 and 3.4 mo in the bortezomib and dexamethasone arms, respectively. Conclusions. Haematological adverse events with bortezomib are predictable and manageable. The kinetics and mechanism appear different from those observed with standard cytotoxic therapy. Neutropenia was transient and rapidly recovered to baseline during the rest period of each bortezomib treatment cycle, with few patients requiring growth factor support. Thrombocytopenia was also transient and reversible. When clinically indicated, platelet transfusion rather than dose reduction or treatment interruption may be warranted to maximise the benefit of bortezomib therapy.

**0494**

**LENALIDOMIDE (REVlimid) COMBINATION WITH DEXAMETHASONE IS MORE EFFECTIVE THAN DEX AMONE IN PATIENTS WITH RELAPSED OR REFRACTORY MULTIPLE MYELOMA AND INDEPENDENT OF NUMBER OF PREVIOUS TREATMENTS**

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Background. Lenalidomide is a novel, oral, immunomodulatory drug that is effective against relapsed or refractory multiple myeloma. In the prospective, randomized, placebo-controlled phase III trial MM-010, lenalidomide with dexamethasone induced a significantly higher response rate and complete remission rate, as well as longer time-to-progression (TTP) in comparison with dexamethasone alone. The trial included patients who had more than 1 previous unsuccessful regimen. Aim. To further investigate whether one or more prior therapies influence the TTP between refractory multiple myeloma in patients treated with lenalidomide and dexamethasone or dexamethasone alone. Methods. This post hoc analyses included 351 patients who had received 1 to 3 prior treatments and were not refractory to dexamethasone. The patients were randomized to receive either oral lenalidomide (25 mg daily for 3 weeks every 4 weeks) plus dexamethasone (40 mg on days 1-4, 9-12, 17-20 every 4 weeks for 4 cycles, then 40 mg on days 1-4 every subsequent cycle) (Len/Dex) or placebo plus dexamethasone (Dex alone). Standard criteria were used to evaluate the median TTP. Confidence intervals (based on Kaplan-Meier estimates), hazard ratios (HR), proportional hazards model, and differences between treatment groups (one-tailed log-rank test of survival curve differences between the treatment groups) were calculated. Results. Of the 351 patients, 4% had previously received bortezomib, 34% had received thalidomide, 67% had received dexamethasone in patients who had received prior thalidomide (8.1% vs. 1.4%, p<0.05). After a median follow-up of 11 months for all patients, there was a trend for Len/Dex to provide improved overall survival (hazard ratio 1.53, p=0.0713) versus Dex alone. Conclusions. Lenalidomide in combination with dexamethasone is more effective than dexamethasone alone in patients with relapsed or refractory multiple myeloma who have received prior thalidomide therapy.

**0493**

**LENALIDOMIDE (REVlimid) IN COMBINATION WITH DEXAMETHASONE IS MORE EFFECTIVE THAN DEXAMETHASONE ALONE IN PATIENTS WITH RELAPSED OR REFRACTORY MULTIPLE MYELOMA WHO HAVE RECEIVED PRIOR THALIDOMIDE THERAPY**

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Background. Lenalidomide is a novel, oral, immunomodulatory drug that is effective against multiple myeloma. In the prospective, randomized, placebo-controlled phase III trials MM-009 and MM-0010, lenalidomide with dexamethasone induced a significantly higher response rate and complete remission rate, as well as longer time-to-progression (TTP) in comparison with dexamethasone alone. The current prospective subgroup analysis was designed to assess whether prior treatment with thalidomide would affect the sensitivity of multiple myeloma to subsequent lenalidomide treatment. Methods. A total of 692 patients who had received 1 to 3 prior treatments and were not refractory to dexamethasone were randomized to receive either oral lenalidomide (25 mg daily for 3 weeks every 4 weeks) plus dexamethasone (40 mg on days 1-4, 9-12, 17-20 every 4 weeks for 4 cycles, then 40 mg on days 1-4 every subsequent cycle) (Len/Dex) or placebo plus dexamethasone (Dex alone). The European Blood and Marrow Transplantation criteria were used to evaluate TTP, complete response (CR), and partial response (PR). Randomization was stratified at entry by number of prior therapies (1 versus >1). Results. Among the 269 patients who had received prior thalidomide treatment, those receiving Len/Dex (N=124) had a longer median TTP (86.9 vs. 19.7 wks, p<0.001) and a higher response rate (CR + PR, 53.2% vs. 15.2%, p<0.001) versus those receiving Dex alone (N=145). Complete response rates were also higher with Len/Dex than with dexamethasone in patients who had received prior thalidomide (8.1% vs. 1.4%, p<0.05). After a median follow-up of 11 months for all patients, there was a trend for Len/Dex to provide improved overall survival (hazard ratio 1.53, p=0.0713) versus Dex alone. Conclusions. Lenalidomide in combination with dexamethasone is more effective than dexamethasone alone in patients with relapsed or refractory multiple myeloma who have received prior thalidomide therapy.
Chronic myeloproliferative disorders

0495
EVIDENCE THAT THE JAK2 V617F MUTATION AND MITOTIC RECOMBINATION OCCUR IN A LYMPHO-MYELOID PROGENITOR CELL IN POLYCYTHEMIA VERA AND IDIOPATHIC MYELOFIBROSIS

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Background. The JAK2 V617F mutation has recently been described as an essential oncogenic event associated with Polycythemia Vera (PV), Idiopathic Myelofibrosis (IMF) and Essential Thrombocythaemia (ET). This mutation has been detected in all myeloid lineages but has not been yet detected in lymphoid cells. This raises the question whether this molecular event occurs in a true lymphoid/myeloid progenitor cell, as it has already been shown that at least some of these myeloproliferative disorders (MPD) arise from a multipotent stem/progenitor cell. Aims. Our aim was to study the presence of the mutation in both myeloid and lymphoid lineages in JAK2 V617F positive MPD. We therefore looked for the mutation first in mature myeloid and lymphoid cells and second in lymphoid/myeloid progenitor cells after CD34+ cell isolation from peripheral blood or bone marrow aspiration. Methods. Ten IMF, 12 PV and 6 ET patients harbouring the mutation were enrolled in the study after informed consent. Peripheral blood granulocytes and platelets were purified by standard methods and B, T, NK cells and monocytes were isolated by combined immunomagnetic and flow cytometric procedures. The same techniques were used to sort CD34+ and CD34+CD38- cells from peripheral blood (IMF patients) or from bone marrow mononuclear cells (PV and ET patients). Clonal B/NK/Myeloid differentiation from CD34+CD38- cells and T cell differentiation from CD34+ cells were performed respectively onto a M55 layer in the presence of SCF, IL7, IL3, IL15, TPO and in murine Fetal Thymic Organ Cultures (FTOC). Genotyping of mature cell populations, B/NK/Myeloid clones and CD34+ derived T cells were performed by sequencing and/or Taqman® real time allelic specific PCR using competitive probes. Results. The JAK2 V617F mutation was present in granulocytes and platelets from all patients, and in monocytes from PV and IMF patients. We detected the mutation in B and NK cells from approximately half IMF patients (respectively 4/7 and 5/8 patients), a minority of PV patients (respectively 1/10 and 1/10 patients), and none of the ET patients. Moreover, 2/8 IMF patients had mutated peripheral T cells whereas none of the PV and ET patients did. The JAK2 V617F mutation could be subsequently detected in CD34+ cells and in B/NK/Myeloid and/or NK/Myeloid CD34+CD38- derived clones from all IMF (n=5), PV (n=5) and ET (n=1) patients, with a much higher frequency in clones derived from IMF. Interestingly, a bi-allelic (homozygous) JAK2 V617F mutation was detected in B/NK/Myeloid and/or NK/Myeloid clones from 2 IMF and 3 PV patients, demonstrating the occurrence of the mitotic recombination in a lymphoid/myeloid progenitor cell. Using the FTOC assay, the mutation was also detected in all T cell fractions derived from CD34+ cells from 2 IMF and 2 PV patients. Conclusions. These results demonstrate that the mutation and the subsequent mitotic recombination leading to a homoygous subclone occur in a lymphoid/myeloid progenitor cell in JAK2 V617F positive MPD. Thus, the phenotype of these MPD arising from a true lymphoid/myeloid progenitor cell may be related to a downstream selective proliferative advantage of the myeloid lineages.

0496
INDOLENT MYELOFIBROSIS WITH MYELOID METAPLASIA: SPECIFIC CLINICAL FEATURES AND PROGNOSIS

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Background. Patients with myelofibrosis with myeloid metaplasia (MMM) have a heterogeneous prognosis, only partially predicted by published scores and parameters. Aims. To identify MMM patients with a survival benefit from aggressive chemotherapy, we have performed a retrospective study of patients selected from intensive chemotherapy regimens. Methods. A prospective national cohort of 871 consecutive patients with MMM was considered. Cluster analysis (EM-algorithm) allowed to classify patients based on their clinical parameters at diagnosis: age, hemoglobin, leukocyte and platelet count, spleen size. Kaplan-Meier survival analysis was applied to the clusters. Results. Five clusters were identified: all showed a significantly different clinical phenotype and survival (p=0.0001). Twenty-nine percent (n=250) of the patients were assigned to the cluster with the highest survival, e.g. indolent MMM. Their median age was 61 years and 38% were females. Twenty-eight percent of the patients with indolent MMM were absolutely asymptomatic, 19% reported a previous essential thrombocytemia and a few polycythemia vera. All the patients with indolent MMM showed at least one of the following features at diagnosis: hemoglobin values >11 g/dl, platelet count >500×10⁹/L, spleen size <4 cm from costal arc. CD34 count <100/mcl. No patient showed circulating blasts and 90% of the patients showed <2% circulating erythroblasts and <10% circulating immature myeloid cells. A specific rule for selecting patients with indolent MMM includes the presence of a limited splenomegaly (<6 cm from costal arc) associated with: a high platelet count (>600×10⁹/L) and/or a normal hemoglobin value and/or an age lower than 65 years. A lower frequency of hematological features was seen in patients with indolent MMM as compared with the rest of the patients (4% vs 31%; p=0.01), irrespetively of previous polycythemia vera. A few patients died of causes directly related to MMM and five-year survival of patients with indolent MMM was 78%. Five-year survival of patients with indolent MMM was higher than overall patients with a low-risk disease, according to the Lille score (p=0.009). Survival did not depend on sex or comorbidity, but depended on age at diagnosis (p=0.0001), five-year survival being >95% in patients aged <40 years, but <75% in patients aged >70 years. Absolute excess mortality, as compared with age-adjusted general population, was 7%, but among patients aged >70 years, absolute excess mortality increased up to 30%. Among patients aged <70 years, females incurred a twice a high excess mortality than males. Percent circulating immature cells was the only clinical parameter that independently predicted survival (p=0.03). Survival was not significantly different in patients followed by a hematology unit as compared with patients followed by an internal medicine unit. Conclusion. A high number of MMM patients disclosed a population with a very good prognosis. Patients with indolent MMM should not be assigned to frontline intensive therapies.

0497
COEXPRESSION OF JAK2 V617F AND TYPE 1 CYTOKINE RECEPTORS IS NOT SUFFICIENT FOR CYTOKINE-INDEPENDENT CELL GROWTH

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Background. An activating point mutation in the JH2 domain of Janus kinase 2 (JAK2) was recently described in chronic myeloproliferative disorders (MPD). The majority of patients with polycythemia vera, and substantial numbers of patients with essential thrombocythemia and idiopathic myelofibrosis carry the JAK2 V617F mutation. Aims. We set out to find cell lines with JAK2 V617F mutation, which may be used as suitable tools to analyze basic aspects of the cell biology of these tumors. It has recently been reported that coexpression of type 1 cytokine receptors with JAK2 V617F proteins leads to cytokine-independence in BA/F3 cells. Our aim was to confirm or refute this correlation in human JAK2 V617F-positive cell lines.

Methods. Cell lines were tested for the JAK2 V617F mutation applying the PCR-based ARMS assay, confirmed by sequencing, and restriction analysis applying the JAK2 wild-type specific restriction enzyme BsaXI.

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Expression and phosphorylation status of JAK2 proteins was checked by immunoprecipitation and Western blot analysis. Cytokine-dependency and influence of JAK kinase inhibitors on cell growth was assayed monitoring 3H-thymidine uptake. Apoptotic cells were detected and quantitated with the annexin-V/propidium iodide method. Results: Five of 19 acute myeloid leukemia-derived cell lines tested expressed the JAK2 V617F mutation and 1/6 (16.7%) AMM patients with clonal granulocytes expressed both mutant (mu) and wild-type (wt) JAK2, the remaining positive cell lines carried homo-/hemizygous JAK2 mutations. Microsatellite analysis confirmed losses of heterozygosity (LOH) affecting the JAK2 region on chromosome 9p in the homozygously JAK2mu cell lines HEL, MB-02, MUTZ-6 and UKE-1. Confirming the importance of the mutated JAK2 protein for growth and prevention of apoptosis, JAK2mu cell lines displayed higher sensitivities to JAK2 inhibition than JAK2wt cell lines. It has recently been reported that JAK2 V617F proteins require coexpression of type I cytokine receptors to secure cytokine-independent activation of the JAK2 and STAT5 pathways and to cause cytokine-independent growth of BA/FS cells. However, 2/3 JAK2mu human AML cell lines described by us were cytokine-dependent, growing after stimulation of type I cytokine receptors: cell line MB-02 responded to erythropoietin and cell line MUTZ-6 responded to G-CSF. Therefore, coexpression of JAK2 V617F and type I cytokine receptors alone is not sufficient for cytokine-independent cell growth. Immunoprecipitation assays showed that HEL, MUTZ-6, and MB-02 cell lines (HEL, SKNO-1, but no JAK2wt cell lines (SKM-1, SKNO-1), exhibited constitutive phosphorylation of JAK2. Also the cytokine-dependent JAK2mu cell line MUTZ-8 showed constitutive JAK2 phosphorylation. However, short-term stimulation with G-CSF induced phosphorylation of JAK2 in cytokine-starved JAK2mu cells, demonstrating that the JAK2 V617F protein was still responsive to G-CSF stimulation. Summary/Conclusions. In summary, our results show (i) that coexpression of JAK2 V617F and type I cytokine receptors is not sufficient for cytokine-independent cell growth and (ii) that JAK2 V617F responds to stimulation of type I cytokine receptors.

**0498**

**ASSESSMENT OF MYELOID CLONALITY IN FEMALE PH-NEGATIVE MYELOPROLIFERATIVE DISORDERS BY JAK2V617F MUTATION AND HUMARA ASSAY**


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**Background.** Essential thrombocytopenia (ET), polycythemia vera (PV) and agnogenic myeloid metaplasia (AMM) are chronic myeloproliferative disorders (MPDs) arising from clonal hematopoietic stem cells. The detection of JAK2 V617F mutation and myeloid clonality by analysis of the human androgen receptor gene (HUMARA), are useful tools to demonstrate clonality in these MPDs. Aim. The aim of this study was to compared two methodologies for clonal hematopoiesis in ET, PV and AMM. Methods. We analyzed a series of female patients with Ph-negative MPDs. However, these biomarkers are still not incorporated in the current ET, PV and AMM diagnostic criteria. Patients and Methods. One hundred and nine females with Ph-negative MPDs (HEV, ET, PV and 7 AMM [9 of them being post-thrombocytopenic myeloid metaplasia]) from a single institution were studied. Patients fulfilled the diagnostic criteria of PVSG for ET and PV and the Italian criteria for AMM. The median age of ET, PV and AMM patients was 64 (range 25-87), 60 (range 25-84) and 57 (range 53-71) years respectively. At the time of HUMARA assessment, 27/65 ET, 12/37 PV and 2/7 AMM patients were receiving therapy with myelosuppressive/platelet lowering agents: hydroxyurea (n=30); anagrelide (n=10) and busulfan (n=1). Twenty-one patients were successfully recognized from IM CD34+ cells. These gene were aberrantly regulated in IM CD34+ cells. The cDNA was hybridized to an Affymetrix HG-U133A oligonucleotide microarray chip representing 22,283 transcripts. By using class prediction analysis, a set of eight gene markers (CD9, G52, DLK1, CD1, WT1, NFE2, HMG4 and CXCR4) was employed to successfully recognize normal from IM CD34+ cells. Methods. For this purpose, we performed a comprehensive transcriptome comparative microarray analysis of normal and IM CD34+ cells. Results. Two hundred sixteen differentially expressed genes were identified; among these, 50 genes that we considered as potentially involved in the pathophysiology of IM were further validated by quantitative RT-PCR. By using class prediction analysis, a set of eight gene markers (CD9, G52, DLK1, CD1H, WT1, NFE2, HMG4 and CXCR4) was employed to successfully recognize normal from IM CD34+ cells. These gene were aberrantly regulated also in the granulocytes of IM, polycythemia vera (PV) and essential thrombocythemia (ET) patients, with some unique patterns; class prediction analysis differentiated IM from normal granulocytes in 100% of cases, while a correct class attribution was obtained in 95% of IM, PV, or ET patients. Altered gene expression was corroborated by the identification of a downregulated gene set in both normal IM, PV, or ET patients. We speculate that the significant down-regulation of CXCR4 in IM CD34+ cells might...
be related to the constitutive mobilization of these cells in the circulation. Abnormal expression of NFE2, HMGA2, and CXCR4 was influenced by the presence of JAK2V617F mutation, unlike WT1, CD9, GAS2, DLK1, and CDH1. There was a direct correlation between expression levels of CD9 and platelet count in IM patients, while DLK1 was inversely related to platelet count. Also, higher WT1 expression levels identified IM patients with more active disease, as evidenced by elevated CD34+ cell count and higher severity score. Conclusions: According to the currently employed clinical-biological parameters, the differential diagnosis among the chronic myeloproliferative disorders may still remain uncertain in a substantial proportion of cases, owing their phenotypic overlapping; therefore, the possibility to use a defined set of molecular markers to approach the diagnosis of IM is of clinical relevance, and has potential diagnostic application. Moreover, determination of WT1 expression levels in IM patients might be suitably employed as the first molecular marker of disease activity and, prospectively, prove useful also to evaluate response to therapy. On behalf of the MPD Consortium.

Antibodies in the treatment of non-Hodgkin’s lymphoma

HUMAX-CD20, A NOVEL FULLY HUMAN ANTI-CD20 MONOClonAL ANTIBODY: RESULTS OF A PHASE I/II TRIAL IN RELAPSED OR REFRACTORY FOLLICULAR NON-HODGKIN’S LYMPHOMA

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Background: The fully human monoclonal IgG1 antibody HuMax-CD20 targets a novel epitope of the CD20 molecule on B-cells. HuMax-CD20 stops growth of engrafted B-cell tumors in SCID mice more efficiently and exerts stronger complement activation than Rituximab. HuMax-CD20 kills Rituximab-resistant cells expressing low levels of CD20. Aims. The objective of the present trial is to establish the safety, efficacy and the pharmacokinetics of HuMax-CD20 in patients with relapsed or refractory follicular lymphoma grade 1-2. Methods. Data are presented from a recently completed, open label, dose-escalation, multicenter clinical trial in patients with relapsed or refractory CD20+ follicular non-Hodgkin’s lymphoma. Four cohorts of 10 patients were treated with 4 weekly i.v. infusions of 300, 500, 700 or 1000 mg. The endpoints include adverse events, centrally reviewed CT verified tumor response according to the Cheson criteria, B-cell depletion, pharmacokinetics and progression free survival. Results. Forty patients have been treated. Mean age was 57 years; median number of prior treatment regimens was 2; 15 patients were previously treated with Rituximab. Rapid, efficient and sustained peripheral B-cell depletion was observed in all dose groups. No dose limiting toxicity has been reported. Only 8 short lasting episodes of grade 3 CTC were observed. Hematological toxicity was low and confined to 6 events of grade 1 neutropenia; no cases of thrombocytopenia were reported. The following pharmacokinetic parameters were derived (medians per dose group): Cmax 129, 185, 380 and 610 µg/mL, T½ 447, 245, 322 and 621 hr, Cl 9, 19, 10 and 7 mL/hr/kg and AUC 75000, 51000, 185000 and 326000 hr µg/mL, for the 300, 500, 700 and 1000 mg dose groups, respectively. No correlation between pharmacokinetics and response was found. Objective responses (CR, CRu, PR) have been evaluated in 37 patients and were obtained in all 4 dose groups; 4 CR + 1 CRu/8 (300 mg), 1CR + 2 PR/9 (500 mg), 2PR/10 (700 mg) and 1 CRu + 4 PR/10 (1000 mg). Objective responses were achieved in 9 of 14 (64%) evaluable patients previously treated with Rituximab, i.e. 3CR, 1 CRu and 5 PR. In total 18 patients showed stable disease; progression was observed in only 3 patients. Based on Kaplan Meier estimates, the median time to progression for all patients was 267 days (95% CI 135-372 days). The median time to progression for responders and the median duration of response have not yet been reached. Conclusions. This final analysis demonstrates a favourable safety profile and encouraging efficacy of HuMax-CD20 in patients with follicular NHL. Objective responses were achieved in all dose groups with response rates up to 63%, including a 64% response rate in patients previously treated with Rituximab. The median time to progression for responders has not yet been reached.
**0502**

ALEMTUZUMAB, FLUDARABINE, CYCLOPHOSPHAMIDE, AND DOXORUBICIN (CAMPATH-FC): AN EFFECTIVE FIRST-LINE TREATMENT IN PERIPHERAL T-CELL LYMPHOMAS


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Background. Peripherally (mature) T-cell lymphomas (PTCL) represent a group of lymphoma entities with an unfavourable outcome after treatment with CHOP or CHOP-like regimens. Aims. The purpose of the study was to investigate the feasibility of a combination of the monoclonal antibody alemtuzumab with chemotherapy consisting of fludarabine, cyclophosphamide and doxorubicin. Methods. Patients were treated with alemtuzumab 3, 10, 30, 50 mg, days 1-4, fludarabine 25 mg/m² days 2-4, cyclophosphamide 600 mg/m² day 3, and doxorubicin 50 mg/m² day 4. Included were patients with primary diagnosis, with first relapse, or with primary refractory disease, excluded were patients with primary cutaneous T-cell lymphomas and AKL-positive large cell anaplastic T-cell lymphomas. Results. So far, 58 patients have been included, 26 evaluable and 12 responders: 12 patients by PTCL, NOS, 9 with AILD, two with ALK-negative ALCI, one with enteropathy-associated T-cell lymphoma, one with NK-cell lymphoma, and one with T-PLL. 15 patients were enrolled with primary diagnosis and 11 patients in relapse. The median age was 58 years (range 21-77); 71% of the patients had an international prognostic index intermediate high or high. In patients with primary diagnosis the CR rate was 67% (10/15), three patients were primary progressive, and two patients dropped out because of treatment associated complications. 9 of the responding patients are in ongoing CR at 2 +, 5 +, 6 +, 13 +, 14 +, 17 +, 20 +, 26+, and 28+ months, respectively. The patient with T-PLL relapsed after being in CR for 25 months. In the group of relapsed or refractory patients two CR and two PR (36% overall response) were observed. The main toxicity was leucopenia (64% grade III and IV of all evaluable treatment cycles), other grade 3 and IV toxicities included anemia (17%), thrombocytopenia (5%), infections (14%), pruritus/skin reactions (9%), nausea/vomiting (6%), mucositis, and cardiac toxicity (5%), two patients with relapsed disease after pre-treatment with CHOP-like regimens developed severe heart failure). 11 (42%) patients reactivated CMV, however, 9 without developing CMV-related disease. Conclusions. The combination is an effective first-line regimen for peripheral T-cell lymphoma, however, regarding the general outcome a longer follow-up period of a larger cohort is necessary. Results of the relapsed/refractory group are promising in relapsed and refractory patients, but is ongoing for first-line treatment of peripheral T-cell lymphomas.

**0503**

GALIXIMAB (ANTI-CD80 MONOCLONAL ANTIBODY) IN COMBINATION WITH RITUXIMAB FOR RELAPSED OR REFRACTIVE, FOLLICULAR NHL: RESULTS OF A PHASE II STUDY


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Background. Galiximab is a monoclonal antibody that targets CD80, a costimulatory molecule that is constitutively expressed on the surface of follicular and other lymphomas. Modest single-agent clinical activity and tolerability were demonstrated in a Phase I study with galiximab in relapsed or refractory, follicular NHL (ORR=11%). Preclinical studies have shown that CD80 and rituximab may exhibit at least additive activity in lymphoma models, supporting the rationale for a combination study. Aims. A Phase I/I study (Study 114-21) evaluating galiximab + rituximab in relapsed or refractory, follicular NHL. Study objectives were to determine safety, pharmacokinetics, and efficacy of the combination regimen. Here we report final results from the Phase II part of the study. Methods. Patients received galiximab (500 mg/m² qwk x 4) with a standard course of rituximab (375 mg/m² qwk x 4). Rituximab refractory patients (no response or a response with TTP <6 months) were excluded. International Workshop Response Criteria were used to evaluate response. Results. Sixty-four patients received treatment. The median follow-up is 20.4 months. Median age at study entry was 59 yrs. Eighty-eight percent of patients were Stage III/IV, with FLIPI low (27%), intermediate (59%), or high (14%) risk groups. All patients had received at least 1 prior lymphoma therapy; 42% were rituximab naive. Galiximab infusions were delivered over 1 hr and were well tolerated. No DLTs were reported. Sixty-one (95%) patients experienced study related AEs; the most common were lymphopenia (44%), leucopenia (58%), fatigue (38%), neutropenia (23%), and chills (23%). An ORR of 64% was observed: 17% CR, 16% CRu, and 31% PR. The median PFS was 12.1 months. Combination therapy did not appear to alter pharmacokinetics. The mean serum half life was 25.7 days for galiximab and 24 days for rituximab. These results were retrospectively compared with 3 historical studies of follicular NHL patients treated with a standard course of rituximab monotherapy. Baseline characteristics were similar; however, there was a higher incidence of rituximab-naive patients in the rituximab monotherapy group (77%) compared with galiximab + rituximab (42%). The toxicity profile of the combination regimen was similar to that observed in the single agent rituximab studies. However, the median PFS was longer in the galiximab + rituximab group (12.1 months) than in the historical rituximab group (10.4 months). In a subset analysis of rituximab-naive patients, the difference in PFS was even more pronounced: 15.4 months in the galiximab + rituximab group vs. 9.4 months in the rituximab monotherapy group. Conclusions. These results suggest that galiximab can be safely combined with a standard course of rituximab, produce promising response rates, and may potentially extend PFS in patients with relapsed or refractory, follicular NHL. A Phase III, randomized, double-blind study is planned.
according to standardized Cheson criteria (reviewed by central radiology), an additional 4-week cycle of MT103 was offered to the patients. **Results.** As of today, 19 patients with a median number of 4 previous chemo-/immuno therapies have been included into MT103-104. During dose-escalation from DL1 (0.5 µg/m²/24h) up to DL3 (5 µg/m²/24h) no dose-limiting toxicity was observed, and AEIs were generally moderate. At DL4 (5 µg/m²/24h on the first day, 15 µg/m²/24h as maintenance dose), 7 patients were treated with 2 patients receiving less than 14 days of treatment. One patient experienced elevation of liver enzymes up to CTC grade 3 after 2 weeks, which recurred upon re-administration of MT103, and 1 patient experienced confusion and disorientation on the second day of treatment. Depletion of circulating B (lymphoma) cells by end of the MT103 infusion was observed in 9 of 15 evaluable patients (with treatment for >2 weeks and B cells detectable in peripheral blood prior to MT103 infusion) with a dose-dependent increase in frequency that reached 100% depletion at DL4. At DL4, 3 of 7 patients had significant bone marrow (BM) infiltration (>10%) with 1 patient showing reduction of and 2 patients showing complete disappearance of lymphoma cells in BM. Best overall tumour response in the 14 evaluable patients (with treatment for >2 weeks and scanning of all involved areas) was 1 CR (at DL4), 2 PR (at DL4), 1 MR, 7 SD and 3 PD. **Summary.** These preliminary results observed in indolent NHL patients clearly indicate single agent biological and clinical activity of MT103.

**OUTCOME FOR PATIENTS WITH DLBCL AND HIGH LDH**

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**Background.** The German High-Grade Lymphoma Study Group (DSHNHL) performed a series of phase II studies evaluating the Mega-CHOEP programme in younger patients (< 60 yrs) with aggressive lymphoma and elevated LDH. Mega-CHOEP consists of 4 courses of intermediate to high-dose chemotherapy (cumulative doses at highest dose level: C: 19.5 g/m², H: 280 mg/m², O: 8 mg, E: 5.04 g/m² and P: 2000 mg) followed by autologous SCT after treatment courses 2-4 (Glass et al., BLOOD 2006 and Schmitz et al. CANCER 2006). Aims. To compare the feasibility, safety, and efficacy of Mega-CHOEP with and without adding 6 infusions of Rituximab (R: 575 mg/m²) on days 1, 14, 35, 56, 77, and 98 of the treatment programme. Methods. The time to treatment failure (TTTF) and overall survival (OS) of all patients with CD20 positive diffuse large-B cell lymphoma (DLBCL) who were treated with Mega-CHOEP, dose level 3 with R (DL3+R) or without R (DL3) were compared using univariate and multivariate analyses. Results. 51 patients were enrolled at DL3 and 59 patients were enrolled at DL3+R. There were no significant differences in stem cell yield, non-hematologic toxicity, or hematopoietic recovery between patients given Mega-CHOEP with or without R. Univariate analysis of TTTF and OS showed significant improvement with the addition of R. With a median follow-up of 2.5 years (Mega-CHOEP + R) and 4.5 years (Mega-CHOEP) OS was 79% with and 55% without R. A multivariate analysis adjusting for the Internationale Prognostic Index (IPI) showed a superior OS for patients given R (relative risk for failure of 2.5 p=0.023). The relative risk for TTTF was 2.4 (p=0.018) when Mega-CHOEP patients treated without or with R were considered. **Summary/Conclusions.** We conclude that in patients with DLBCL the addition of R to dose escalated CHOEP plus Etoposide (Mega-CHOEP) significantly improves OS and TTTF without adding significant toxicity. The DSHNHL is currently running a prospective randomized trial comparing R-Mega-CHOEP with 8 courses of R-CHOEP given every 2 weeks in young high-risk patients with aggressive lymphoma.
0506
TRISOMY 8 IS ASSOCIATED WITH A HIGHER EXPRESSION OF A SUBSET OF GENES LOCATED ON CHROMOSOME 8 DETERMINED BY THE ACCOMPANYING GENETIC ABNORMALITIES

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Background. Trisomy 8 is the most frequently observed trisomy in AML occurring as a sole karyotype abnormality or in addition to other chromosome aberrations. 2. Aim. It was the aim of this study to analyze the impact of trisomy 8 on the expression of genes located on chromosome 8 in different AML subgroups. 3. Methods. Gene expression analyses were performed in a total of 567 AML cases. The following 14 subgroups were analyzed: +8 sole (n=19), +8 within a complex aberrant karyotype (n=11), +8 with t(15;17) (n=7), +8 and inv(16) (n=6), +8 with t(8;21) (n=3), +8 and 11q23/MLL (n=6), and +8 with other abnormalities (n=10). These were compared to 200 AML with normal karyotype and the following subgroups without trisomy 8: complex aberrant karyotype (n=73), t(15;17) (n=86), inv(16) (n=46), t(8;21) (n=57), 11q23/MLL (n=57), and other abnormalities (n=77). 4. Results. A significant higher mean expression of genes located on chromosome 8 was observed in subgroups with +8 in comparison to their respective control groups. A varying number of significantly higher expressed genes was identified in all comparisons. No gene was significantly overexpressed in all comparisons. No distinct gene expression pattern was identified allowing the identification of cases with trisomy 8. Therefore, the gain of chromosome 8 might reflect a higher expression of genes located on chromosome 8. However, no consistent pattern of genes was identified which shows a higher expression in all AML subtypes with trisomy 8. 5. Summary / Conclusions. This data suggests that trisomy 8 rather provides a platform for a higher expression of chromosome 8 genes which are individually upregulated by the respective primary genetic abnormalities. Therefore, trisomy 8 in AML determines no specific disease characteristic but is a disease modulating secondary event.

0507
JAK2 AND FLT3 MUTATION SCREENING IN 256 PATIENTS WITH AML, MDS AND A NORMAL KARYOTYPE. THE PRESENCE OF V617F CORRELATES WITH A HIGHER FREQUENCY OF CRYPTIC GENETIC ABERRATIONS

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AML and MDS are clinically and molecularly heterogeneous diseases. Karyotype is one of the most important prognostic factors. Effective risk stratification is especially difficult for patients with normal karyotype, which account for more than 40% of all cases. In AML, the clinical outcome of patients with normal cytogenetics can be predicted by the presence or absence of mutations or by changes in the expression of specific genes. Mutations in FLT3 are detected in 30% of AML and in 5-5% of MDS, and are associated with unfavorable prognosis. The V617F activating mutation of JAK2 has been recently described as a common event in MFD, and has been found in a small number of patients with either AML or MDS. Although there are few studies, the incidence of JAK2 mutation seems to be similar in both groups (3%). However, apart from CML (5.8%), there are no data about its relationship with either specific MDS subgroups or with the karyotype. More still, a detailed correlation between mutant JAK2 and FLT3 has yet been fully addressed. In this study we investigated the incidence of JAK2 and FLT3 mutations in 176 patients with AML and 80 MDS, with normal karyotype, and its relationship with cryptic genetic aberrations analyzed through a SNP array platform. V617F genotyping was performed by ARMS as previously described (Jones et al., 2006). All cases tested positive were confirmed by sequencing. A high resolution 50K SNP array (Affimetrix) was used to analyze 14 patients. We found the mutation V617F in 10 cases: 6 AML (5.4%) and 4 MDS (5%), confirming the low incidence of this mutation in AML and MDS. The frequency in the FAB subgroups was 2% in M1 (2/105), 10.8% in M2 (4/38), 5.8% (2/35), and 11% (5/45) in M4 (3/27). As expected, 30% of AML had FLT3 mutations, and only 2.5% of the MDS patients (2/80). Of the 28 CML patients analyzed, one had V617F (3.5%), and 2 ITD-FLT3 (7.7%), suggesting that it would be interesting to analyze FLT3 mutations in MPD patients with no JAK2 mutations. As previously reported, the SNP array technology permits the simultaneous analysis of copy number and allelotype data. Using the 50K SNParray, cryptic genetic aberrations were identified in the 14 patients analyzed. We found 13 recurrent amplifications in the mutated cases. In conclusion, we found that more than 30% of the cases with normal karyotype had regions of LOH by UPD. The SNP array technology could be a useful method to define subgroups in patients with normal karyotype, and we found a higher frequency of cryptic aberrations in cases with V617F. The incidence of the mutation V617F is low in both AML and MDS, although prospective studies are needed to confirm the definitive role of these mutations in the patients prognosis.

0508
GENETIC PATHWAYS OF THERAPY-RELATED MDS AND AML (T-MDS/T-AML)

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Background. t-MDS/T-AML may serve as a model for leukemic transformation. Aims. to relate cytogenetic characteristics to mutation of eleven different genes in t-MDS/t-AML. Methods. 89 patients with t-MDS/t-AML were studied by standard Methods. Results. Eight different genetic pathways were identified. Pathway I included patients with t-MDS and 7q/-7 but normal chromosomes 5 and without balanced translocations. Point mutations of AML1 and methylation of the p15 promoter were common in this pathway. Pathway II included patients with t-MDS and 7q/-7 with or without abnormalities of chromosome 7. In this pathway 77% presented p53 mutations often combined with deletions of 17p, complex karyotypes and complicated chromosome rearrangements. Sometimes amplification of 11q23 or 21q22 was observed. Most patients in pathway I and II had received alkylating agents. Pathway III included patients with t-AML and translocations of 11q23. Pathway IV included patients with balanced chromosome aberrations involving 21q22 or 16q22. Except for cases with t(3;21) these patients presented a t-AML. Patients with involvement of 21q22 often had additional 7q/-7 and occasionally c-KIT or FLT3 mutations. Pathway V included patients with t-AML and translocations to 17q12. They sometimes presented FLT3-ITD. Pathway VI included cases of t-MDS or t-AML and balanced translocations to 11p15 and rearrangement of the NUP98 gene. Most patients in pathway III-VI had previously received topoisomerase II inhibitors. Pathway VII included patients with a normal karyotype often presenting as t-AML and 50% had mutations of FLT3-ITD, RAS or AML1. Pathway VIII included patients with mutations of FLT3-ITD and RAS in combination. Mutation of JAK2 was observed in only two atypical cases of t-MDS in this pathway. Pathway VIII included patients with atypical chromosome rearrangements and only 2/20 in this pathway presented RAS mutations. A significant association was observed between mutations of genes in the RAS/RAS-BRAF signalling pathway (n=55) and mutation or rearrangement of genes for putative transcription factors (n=49), so called class I and II mutations, as 18 patients, 15 belonging to pathways III-V and VII, presented both types of mutation (p=0.012, Fishers exact test, two sided). Conclusions. classification of t-MDS/t-AML in different genetic pathways is supported and an association between class I and II mutations is confirmed.

0509
NEW STRATEGY TO IDENTIFY TUMOR SUPPRESSOR GENES IN ACUTE MYELOID LEUKEMIA

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Background. Retroviral integration mutagenesis in mice is a powerful tool to discover novel genes involved in the development of leukemia. Using the Grifiti 1.4 murine leukemia virus (Gr-1.4 MuLV), we identified candidate disease genes of acute myeloid leukemia (AML) (Erkeland et al., J Virol. 2004; 78: 1971-80). Recently, we reported that genes adjacent to the virus integration site (VIS), so-called VIS genes, contribute significantly to gene expression profiles of distinct subgroups of human AML, supporting the importance of deregulation of VIS genes in the pathogen-
ties among different tumor samples. Distinct methylation categories were defined: high (n=7), medium-high (n=15), medium (n=12), low (n=20) and none (n=27). Enrichment of LTRs after MeDIP with a5-mC was found in 25/34 samples. As expected, MeDIP on normal hematopoietic tissues was negative for LTR methylation status among different tumor samples. These gene products include known suppressor genes such as Smad1 and Mad1-like, as well as a number of genes with as yet poorly characterized roles in cancer. Summary/conclusions. We present a new strategy to identify tumor suppressor genes in AML. The marked variability in DNA methylation of VIS in different tumor samples indicates that most viral integrations occur in non-methylated parts of the DNA, otherwise the DNA methylation of VIS in different tumor samples would be more equal. The potential tumor suppressor genes in these regions, which may be silenced through methylation spreading, will be identified by direct PCR strategies in combination with bisulfite treatment. To test the relevance of these genes for clinical disease, their expression will be analyzed in a large cohort of AML patients, of which gene expression profiles are already available.

Aim

Methods.

Results.

Conclusions.

Background. Oral contraceptive use is a risk factor for ischemic stroke in young women. While hyperhomocysteinemia increases the risk of the disease, the role of inherited thrombophilia is still uncertain. Little data exists on the interaction between such risk factors and the risk of ischemic stroke in young women. Aims. To assess the interaction between thrombophilia and oral contraceptive intake in determining the risk of ischemic stroke in young women. Methods. One-hundred and five women with a first ischemic stroke at an age less than 45 years and 293 healthy controls were investigated for the presence of thrombophilia due to factor V Leiden, prothrombin G20210A mutation, antithrombin, protein C and protein S deficiency, and hyperhomocysteinemia. The presence of oral contraceptive use was recorded. Fifty-five women had a stroke of undetermined origin. Results. Oral contraceptives were associated with an increased risk of ischemic stroke (odds ratio 2.3, 95% CI 1.4-3.8) in the first 6-18 months of use. The risk of ischemic stroke was also higher in patients with hyperhomocysteinemia (odds ratio 3.5, 95% CI 1.9-6.4), in those with factor V Leiden (odds ratio 2.6, 95% CI 1.0-6.8), but not in those with prothrombin G20210A (odds ratio 0.9, 95% CI 0.1-11.2). After stratification for the presence of oral contraceptive use and thrombophilia due to factor V Leiden or hyperhomocysteinemia, the odds ratio for ischemic stroke in women with both risk factors was 12.9 (95% CI 1.3-138.7) for factor V Leiden and 9.2 (95% CI 2.5-33.8) for hyperhomocysteinemia. No increased risk was observed when oral contraceptive use and prothrombin G20210A were present together. The risk associated with oral contraceptive use, hyperhomocysteinemia, or both, was more pronounced for stroke of undetermined than determined etiology. Conclusions. The use of oral contraceptives is associated with a 2-fold increased risk of ischemic stroke. This risk quintuples in the presence of hyperhomocysteinemia or factor V Leiden. These findings should be taken into account for thrombophilia screening after an ischemic stroke and for individual decision making when prescribing oral contraceptives.

Aims.

Methods.

Results.

Conclusions.

Background. Deep venous thrombosis (DVT) and pulmonary embolism (PE) are amongst the most common disorders in developed countries. Only in about 50% of patients with a history of unprovoked venous thromboembolism (VTE) a genetic or acquired risk factor can be specified. Experimental, epidemiological and clinical studies indicate an association between serum lipids and VTE. Hyperlipidemia and overweight may play a role in the development of VTE by influencing the homeostasis of the clotting and fibrinolytic system and could thereby induce a hypercoagulable state. Aims. The aim of our present study was to elucidate a possible association of lipids and overweight with VTE in high risk patients, who suffered from objectively confirmed recurrent VTE. Methods. We conducted a case-control study to analyse the relationship between serum lipids and the risk of VTE. Outpatients with a history of objectively confirmed recurrent VTE, who had at least one event of an unprovoked DVT or PE, were recruited from 01/2005 to 11/2005. Age and sex-matched healthy individuals served as controls. Venous blood samples were obtained after overnight fasting for serum lipid determinations (total cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL) and triglycerides). Height (m) and weight (kg) were recorded and body mass index (BMI) was calculated (kg/m²). Hyperlipidemia was diagnosed when serum cholesterol level was over 200 mg/dL, triglyceride level over 172 mg/dL or cholesterol/HDL-quotient > 4. A BMI above 24.99 kg/m² was characterized overweight. Mann-Whitney-U test was carried out to compare the groups. Univariate logistic regression analyses were applied to calculate odds ratios and the 95% confidence interval. Results. Hundred-sixteen patients (58 female / 68
male; mean age 56 ±12 yrs) with a history of recurrent VTE and 129 age and sex-matched controls (66 female / 63 male; mean age 53 ±11 yr) were enrolled. Patients showed a significantly higher BMI than controls (median (Md) 27.45 kg/m² vs 25.78 kg/m², p=0.032). Total cholesterol (Md = 235 mg/dL vs. 230 mg/dL, p=0.22) and LDL (Md = 147 mg/dL vs. 141 mg/dL, p=0.074) serum levels were not significantly different. HDL levels were significantly lower in patients (Md = 56 mg/dL vs. 60 mg/dL, p=0.085) and the cholesterol/HDL-quotient significantly higher (Md = 4.24 vs. 3.66, p=0.001). Triglyceride levels also differed significantly (Md = 135 mg/dL vs. 111 mg/dL, p=0.006). Estimated odds ratios for VTE were 1.86 (95% CI, 1.08-3.19; p=0.02) for hypercholesterolemia (95% CI, 0.72-2.40; p=0.074) serum levels were not significantly different. HDL levels were significantly lower in patients (Md = 56 mg/dL vs. 60 mg/dL, p=0.085) and the cholesterol/HDL-quotient significantly higher (Md = 4.24 vs. 3.66, p=0.001). Triglyceride levels also differed significantly (Md = 135 mg/dL vs. 111 mg/dL, p=0.006). Estimated odds ratios for VTE were 1.86 (95% CI, 1.08-3.19; p=0.02) for overweight, 1.32 for hypercholesterolemia (95% CI, 0.72-2.40; p=0.074) serum levels were not significantly different. HDL levels were significantly lower in patients (Md = 56 mg/dL vs. 60 mg/dL, p=0.085) and the cholesterol/HDL-quotient significantly higher (Md = 4.24 vs. 3.66, p=0.001). Triglyceride levels also differed significantly (Md = 135 mg/dL vs. 111 mg/dL, p=0.006). Estimated odds ratios for VTE were 1.86 (95% CI, 1.08-3.19; p=0.02) for overweight, 1.32 for hypercholesterolemia (95% CI, 0.72-2.40; p=0.074) serum levels were not significantly different. HDL levels were significantly lower in patients (Md = 56 mg/dL vs. 60 mg/dL, p=0.085) and the cholesterol/HDL-quotient significantly higher (Md = 4.24 vs. 3.66, p=0.001). Triglyceride levels also differed significantly (Md = 135 mg/dL vs. 111 mg/dL, p=0.006). Estimated odds ratios for VTE were 1.86 (95% CI, 1.08-3.19; p=0.02) for overweight, 1.32 for hypercholesterolemia (95% CI, 0.72-2.40; p=0.074) serum levels were not significantly different. HDL levels were significantly lower in patients (Md = 56 mg/dL vs. 60 mg/dL, p=0.085) and the cholesterol/HDL-quotient significantly higher (Md = 4.24 vs. 3.66, p=0.001). Triglyceride levels also differed significantly (Md = 135 mg/dL vs. 111 mg/dL, p=0.006). Estimated odds ratios for VTE were 1.86 (95% CI, 1.08-3.19; p=0.02) for overweight, 1.32 for hypercholesterolemia (95% CI, 0.72-2.40; p=0.074) serum levels were not significantly different. HDL levels were significantly lower in patients (Md = 56 mg/dL vs. 60 mg/dL, p=0.085) and the cholesterol/HDL-quotient significantly higher (Md = 4.24 vs. 3.66, p=0.001). Triglyceride levels also differed significantly (Md = 135 mg/dL vs. 111 mg/dL, p=0.006). 

Methods. Consecutive patients, with an episode of idiopathic or provoked DVT, were evaluated after 3 months from the index DVT. The presence/absence of RVT was detected and patients managed consequently; in those with RVT, OA was continued for 1 year while in those without RVT, OA was discontinued. The incidence of VTE recurrence, overt cancer and new CD was evaluated over a period of almost 3 years after the index DVT. Survival curves (Kaplan-Mayer) and related Breslow test have been used for statistics. Results. Three-hundred forty-five patients were included in the analysis. The results are listed in the figures. The incidence of recurrent VTE and new overt cancer was statistically lower in patients without RVT than in those with RVT; no significant differences were found in the incidence of new CD. These data are applicable in patients with idiopathic or provoked index DVT. In patients with RVT, the advantage of prolonging anticoagulation for 12 months is lost at the end of the treatment. Conclusions. This is the first study evaluating the relationship between US-detected RVT and the risk of developing cancer and CD; RVT persistence, at 3rd month from the index DVT, is an independent risk factor for recurrent VTE and indicates patients at risk for new overt cancer. This risk remains over a period of almost 3 years, independently whether index DVT was idiopathic or provoked. In these patients, the advantage of indefinite anticoagulation should be assessed in properly designed study.

0512

THE PERSISTANCE OF RESIDUAL VEIN THROMBOSIS, AFTER AN EPISODE OF DEEP VEIN THROMBOSIS, AND THE RISK OF NEW OVERT CANCER AND CARDIOVASCULAR DISEASE

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Background. We have recently demonstrated that the presence of Residual Vein Thrombosis (RVT), Ultrasoundography (US)-detected at the 3rd month after an episode of Deep Vein Thrombosis (DVT) of the lower limbs, is an independent risk factor for developing recurrent Venous Thromboembolism (VTE). The management of DVT patients by detection of RVT may, therefore, represent a simple and reproducible method for establishing the individual risk of recurrence and for tailoring the optimal duration of Oral Anticoagulants (OA) (Siragusa S et al. Blood 2003;102(11):OC183a). At the present, it is unknown whether RVT may also identify patients at increased risk for cancer and/or cardiovascular disease (CD). Objective of the study. In patients with DVT of the lower limbs, we conducted a prospective study for evaluating the correlation between RVT and the risk of new overt cancer and CD. Materials and Methods. Consecutive patients, with an episode of idiopathic or provoked DVT, were evaluated after 3 months from the index DVT. The presence/absence of RVT was detected and patients managed consequently; in those with RVT, OA was continued for 1 year while in those without RVT, OA was discontinued. The incidence of VTE recurrence, overt cancer and new CD was evaluated over a period of almost 3 years after the index DVT. Survival curves (Kaplan-Mayer) and related Breslow test have been used for statistics. Results. Three-hundred forty-five patients were included in the analysis. The results are listed in the figures. The incidence of recurrent VTE and new overt cancer was statistically lower in patients without RVT than in those with RVT; no significant differences were found in the incidence of new CD. These data are applicable in patients with idiopathic or provoked index DVT. In patients with RVT, the advantage of prolonging anticoagulation for 12 months is lost at the end of the treatment. Conclusions. This is the first study evaluating the relationship between US-detected RVT and the risk of developing cancer and CD; RVT persistence, at 3rd month from the index DVT, is an independent risk factor for recurrent VTE and indicates patients at risk for new overt cancer. This risk remains over a period of almost 3 years, independently whether index DVT was idiopathic or provoked. In these patients, the advantage of indefinite anticoagulation should be assessed in properly designed study.

0513

REGULATION OF PROTEIN S EXPRESSION BY SEX HORMONES

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Background. The anticoagulant Protein S (PS) is coded for by the PROS1 gene and serves as a co-factor to APC inactivation of FVa and FVIIIa. Previous studies have shown a reduction in circulating PS levels in response to increasing oestrogen (E2) levels resulting in an increased thrombotic risk. This relationship is evident in women who are pregnant or are using oral contraceptives (OCs). To date, a mechanism to describe this relationship at the molecular level has not been elucidated. We have identified a potential oestrogen response element (ERE) spanning nucleotides -850 to -867 within the promoter region of PROS1. Aims. To devise a method that quantitatively measures the expression of the PROS1 promoter and use it to study the effect of E2. Methods. Using an EGFP expression vector, clones containing 950bp of the PROS1 promoter up to, but not including the ATG codon and which included the ERE motif, were transiently transfected into the breast carcinoma cell line, MCF-7. Levels of EGFP were measured by flow cytometry following exposure to E2, with and without excess oestrogen receptor α (ER). Progestins form the second component of most OCs, therefore the same experiment was performed with progesterone (P4). Co-transfection’s with excess progesterone receptor’s A and B (PRA and PRB) were also assessed. Results. Reflecting clinical observations the expression of the PROS1 promoter fragment decreased in response to E2 and was further reduced in the presence of excess ER. Interestingly, the opposite was seen in response to P4. Up-regulation via P4 was further increased in the presence of excess PRB, but not PRA. Summary/Conclusions. These results show that PROS1 promoter expression is reduced in the presence of E2. The down-regulation is enhanced by ER, suggesting that the effect is mediated via an ER, dependent mechanism. However, the promoter region is also responsive to P4 which up-regulates expression in what appears to be a mechanism involving PRB. The opposing effects seemingly counterbalance each other. Based on these results the attention given to the oestrogen component of OCs may not be as important as the progestin component. It is the progestin that varies between different OC preparations and not the oestrogen which is predominantly ethynyl oestriodial. Thus, the increasing evidence that 3rd generation OCs represent a greater thrombotic risk when compared to 2nd generation formulations, could be more about the types of progestins used in the 3rd generation OCs, and their ability to counteract the effect of ethynyl oestriodial.
Rituximab causes a reduction in IgG antibodies to ADAMTS 13 and promptly to Rituximab and require significantly fewer PEX. In addition, requirement for PEX. Our results suggest patients with TTP respond promptly to Rituximab, with a significant reduction in the malisation of CD 19 levels, between 6-15 months has not been associ-
those likely to have IAA but who had isolated clinical positive findings which could also be present in FA [n=16, group-3]. Patients with a known or obvious FA diagnosis were not included in the study. Chromosome breakage test and FANCD2 immunoblot were performed in PBL in all patients [n=65]. Also, skin primary fibroblasts were analysed [n=40] to detect potential haematopoietic FA reversion. Because chromosome breakage test is rarely efficient in fibroblasts, we performed FANCD2 immunoblot and developed a new flow cytometry test based on MMC-sensitivity in fibroblasts to identify FA/BRCA downstream groups. Results. In total, 4 patients with FA were identified. The only positive clinical findings for those patients were: Patient-1, group-3 (precocious menopause, vocal cord neoplasia at age 38yo), Patient-2, group-3 (BMF at age 10yo following a period of isolated thrombocytopenia, 1 café-au-lait spot hypoplasias), Patient-3, group-2 (low birth weight/short stature, ‘peculiar’ facies, onset of pancytopenia only at age 25yo) and Patient-4, group-1 (10yo, no positive clinical findings; did resemble IAA). The two patients from groups 1 and 2 were diagnosed with chromosomal breakage test, and further classified with FANCD2 immunoblot in PBL. For the two patients from group-3, based on persistent clinical suspicion of FA after negative breakage test in PBL, somatic mosaicism with complete haematopoietic reversion was diagnosed using FANCD2 immunoblot fibroblast analysis. Importantly, FA diagnosis was definitely excluded in all other patients. Conclusions. In situ analyses where the suspicion of FA persists after a negative breakage test in PBL (e.g. congenital physical abnormalities and possible mosaicism), then diagnostic tools should be performed on fibroblasts. As a rule, we found that underdiagnosing FA is very rare if careful history and physical exam are done together with standard chromosome breakage tests in PBL. Because no cases of FA were found in our cohort of patients with IAA presentation and negative breakage test, we suggest that screening can be limited to this technique. The strategy here presented allowed us to identify a few unexpected FA cases in a cohort of BMF patients, and importantly, to definitely rule out FA in others.

0517

THE EFFECT OF COMBINED THERAPY WITH DEFEROXAMINE AND DEFERIPRONE ON MYOCARDIAL IRON AND ENDOTHELIAL FUNCTION IN THALASSEMA MAJOR: A RANDOMIZED CONTROLLED TRIAL USING CARDIOVASCULAR MAGNETIC RESONANCE

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Background. In β-thalassemia major (TM) cardiac failure secondary to myocardial iron loading remains the leading cause of death. Approximately one-third of patients maintained on deferoxamine continue to exhibit myocardial iron loading. The oral iron chelator, deferiprone has been demonstrated to remove myocardial iron and it has been proposed that in combination with deferoxamine it may have an additive effect. Myocardial iron can be rapidly and reproducibly quantified using a cardiac magnetic resonance (CMR) T2* technique. Endothelial function (as quantified by flow mediated dilatation of the brachial artery (FMD)) can be impaired in TM which may further contribute to cardiovascular pathology. FMD is also reliably measured by CMR. CMR is therefore well suited to assess the efficacy of new therapies for the treatment of iron overload in TM. Aims. To report the changes in myocardial iron loading (changes in T2*) and endothelial function (as assessed by FMD) from a randomized placebo controlled trial comparing the combined therapy of deferiprone and deferoxamine with the standard therapy of deferoxamine alone. Methods. A mobile CMR scanner (1.5T, Siemens Sonata) was transported to Cagliari, Italy. The myocardial T2* was assessed in 167 patients with TM. 65 patients (male 27, female 38, age 29±4.8years) with mild-moderate cardiac iron loading (T2* 8-20ms) were randomized to receive either deferiprone and placebo, or deferoxamine and deferiprone. Myocardial and hepatic T2* were assessed at baseline, 6 and 12 months. Endothelial function was assessed at 0 and 12 months. Results. Analysis of covariance showed a significant difference between the two groups, with the combined group showing superior effects in reducing both myocardial iron (p=0.017) and hepatic iron (p<0.001). See figure 1. Over the 12 months endothelial function improved significantly in the combined treatment group (from 10.5% to 13.8%, p=0.001) but not in the placebo control group (9.9% to 13.4%, p=0.10). Conclusions. In patients with mild-moderate cardiac iron loading the combined therapy of deferiprone and deferoxamine is superior to deferoxamine alone in the removal of myocardial iron and improving endothelial function.

Figure 1. Both heart and liver iron loading significantly improve with combined chelation therapy (continuous line). There is no significant improvement in the placebo group (dashed line).

0518

PAROXYSMAL NOCTURNAL HAEMOGLOBINURIA: LONG-TERM EPIDEMIOLOGICAL STUDY

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Background. Paroxysmal nocturnal haemoglobinuria (PNH) is a rare acquired disorder of haematopoietic stem cells. Although knowledge about the pathophysiology of the disease is increasing, few studies have been published on the long-term follow up mainly because of the rarity of the disease. Aims. Analyzing a large cohort of patients with PNH on the long term to better determine prognostic factors. Assessing the role, if any, of the introduction of flow cytometry for diagnosis in the presentation and following of the disease. Methods. We have already reported such an analysis on 220 patients in 1996 (Socié et al., Lancet). Data were updated and collected on an additional 285 patients with PNH. Haematological centres were contacted by the way of the French Society of Haematology and/or the French Association of Young Haematologist. The date of diagnosis was based on blood cytometric analysis if there was no prior positive Ham’s test. Data validation is still in progress. Results. Provisory results are the following. We report here the natural history of PNH among 478 patients (258 female, 220 male). 58 French haematological centers participated in the study. Patients were diagnosed over a 55-year period (1950-2005). The age at diagnosis was 34 (inter-quartile range: 24-47). The median follow up (+ standard deviation) is 5.6 years (+0.4). 50 patients underwent allogeneic bone marrow transplantation. During the evolution, 113 patients presented a thrombosis, 9 a myelodysplasia, and 6 an acute leukemia, respectively. Ninety six patients died. The Kaplan-Meier survival (± standard deviation) was 85% at 5 years (±2), 76% at 10 years (±3), and 66% at 15 years (±3). The analysis of prognostic factors are on going at time of abstract submission. Conclusion. This is the largest cohort of patients with PNH reported until now. Definitive results after complete validation of the database will be presented at the meeting.

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HIGH PREVALENCE OF PULMONARY HYPERTENSION AND HEMOLYSIS ASSOCIATED WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

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Background. Pulmonary hypertension (PHT) is an emerging common complication of hereditary hemolytic anemias. It has been mechanistically and epidemiologically linked to intravascular hemolysis and decreased nitric oxide (NO) bioavailability. The release of excessive red cell hemoglobin during intravascular hemolysis can exceed the capacity of the hemoglobin scavenging molecule, haptoglobin, leading to the consumption of endogenous NO. While this complication has been described in approximately 30% of adult patients with sickle cell disease and thalassemia, the prevalence of PHT in patients with paroxysmal nocturnal hemoglobinuria (PNH), an acquired disease with the highest levels of intravascular hemolysis observed, has never been determined. PNH patients frequently have symptoms consistent with both hemolysis and PHT including severe fatigue and dyspnea on exertion. Aims. We, therefore, examined for the presence of PHT in PNH and explored potential mechanisms associated with its development by measuring the ability of plasma to instantaneously consume NO. Methods. Doppler echocardiography was performed in 28 hemolytic PNH patients to estimate pulmonary artery pressures. Transmural flow, Doppler determinations of systolic pressure, and left ventricular stroke volume were assessed and graded. Systolic PHT was prospectively defined by a tricuspid regurgitant jet velocity (TRV) of at least 2.5 m/s. Nitric oxide consumption was assessed using ozone-based chemiluminescence, and red cell hemolysis was determined by plasma levels of lactate dehydrogenase (LDH). Blood was collected using methodologies to limit artefactual hemolysis. Results. Tricuspid regurgitation was observed in 20 out of 28 patients with PNH. Fourteen of these 20 evaluable patients (70%) demonstrated elevated pulmonary artery systolic pressures. Twelve (60%) had mild to moderate PHT (mean TRV 2.6 m/s±0.1) while two (10%) had moderate to severe pressures (mean TRV 3.7 m/s±0.2). Plasma from PNH patients (n=32) consumed 54±8.3 micromolar NO while normal subjects (n=9) consumed 2.2±0.6 micromolar NO (p<0.0001). LDH levels correlated with NO consumption (r=0.634, p=0.0002). Eculizumab is a humanized monoclonal antibody that binds to C5 inhibiting terminal complement activation. In a separate cohort of 7 patients treated with eculizumab for a median of 3 years to reduce hemolysis, the ability to consume NO appeared lower (19.2±4.8 micromolar NO). Conclusion. This study shows, for the first time, that IL-7-/- mice have a robust regulatory FoxP3-expressing CD4+ T cell compartment that controls T-cell mediated disease. It also highlights the potential of regulatory FoxP3-expressing CD4+CD25+ T cell population to restore a functional CD4+CD25+ T cell compartment through an IL-7 independent pathway.

LYSOSOMAL ROUTING OF G-CSF RECEPTORS DEPENDS ON A SINGLE MEMBRANE-PROXIMAL LYSINE RESIDUE, IS CONTROLLED BY SOCS3 AND PLAYS A CRITICAL ROLE IN G-CSF-INDUCED GRANULopoIESIS

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Background. The G-CSF receptor (G-CSF-R) tightly controls proliferation, survival and differentiation of myeloid progenitor cells. Mutations truncating the C-terminus of G-CSF-R are found in severe congenital neutropenia (SCN) patients at risk to develop AML. Myeloid progenitor cells expressing truncated G-CSF-R hyperproliferate in response to G-CSF and show defective differentiation. These truncated G-CSF-R have lost their recruitment site for SOCS3 and are hampered in receptor internalization. Aims. To study the role of receptor internalization and postendocytic routing in the control of G-CSF signaling and to determine the underlying mechanisms of these processes. Methods. We studied internalization rate, post-endocytic trafficking, signal transduction and proliferation/differentiation characteristics of various mutant G-CSF-R. Results. We investigated whether the absence of disease was due to the fact that IL-7 is dispensable for the ontogeny, function and homeostasis of regulatory Foxp3- expressing CD4+ T cells. Alternatively, the absence of IL-7 might directly prevent manifestations of T-cell mediated disease. Methods. Frequencies of CD4+CD25+ T cells in the thymus and spleen of IL-7-/- mice were assessed by Flow Cytometric analysis. We studied the expression of Foxt3 in thymic and peripheral CD4+CD25+ T cells in IL-7-/- mice. The potential of pathogenic and regulatory IL-7-/- T-cells was evaluated in co-transfer experiments. Finally, CD25+CD4+ T cells in IL-7-/- and control mice were depleted by 10 daily injections of PC61mAb (anti-CD25), and microscopic analysis of the colon was subsequently carried out. Results. We show here that the establishment of the peripheral pool of Foxp3- expressing regulatory CD4+CD25+ T cells is IL-7 independent, and the premature involution of the thymus in IL-7-/- mice does not change the representation of CD4+CD25+ T cell compartment. The frequency of peripheral activated CD4+ T-cells increases with age in both CD25- and CD25+ compartments, with CD4+CD25+ T-cells displaying signs of constant activation. IL-7-/- CD4+CD25+ T cells control Inflammatory Bowel Disease induced by IL-7-/- CD25-CD45RBBright T cells, even in hosts lacking IL-7. Depletion of CD25+ T cell subset after thymic involution results in a mild form of IBD, which resolves concomitantly with the regeneration of this subset. Conclusion. This study shows, for the first time, that IL-7-/– mice have a robust regulatory Foxp3-expressing CD4+ T cell compartment that controls T-cell mediated disease. It also highlights the potential of regulatory Foxp3-expressing CD4+CD25+ T cell population to restore a functional CD4+CD25+ T cell compartment through an IL-7 independent pathway.
TRANSLATION OF IGBP1 mRNA CONTRIBUTES TO THE REGULATION OF EXPANSION AND DIFFERENTIATION OF ERYTHROID PROGENITORS

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Background. Erythroid progenitors can be expanded in vitro in the presence of erythropoietin (Epo) and stem cell factor (SCF), while they differentiate to enucleated erythrocytes in presence of Epo only. Previously we showed that SCF-induced delay of differentiation depends on the activation of phosphoinositide-3-kinase (PI3K). An important PI3K-dependent process in cell proliferation is regulation of mRNA translation. PI3K controls the activity of mTOR (mammalian target of rapamycin), whose activation results in phosphorylation of eIF4E-binding protein (4E-BP). Fully phosphorylated 4E-BP releases eIF4E, the scaffolding protein of the eIF4F cap-binding complex. In particular mRNAs with a structure resembling the 4E-BP binding region (4E-BP) require optimal availability of eIF4E to be translated. SCF, but not Epo can induce full phosphorylation of 4E-BP and efficient formation of the 4E-BP binding complex. Overexpression of eIF4E inhibited erythroid differentiation, indicating that SCF-induced formation of the eIF4F complex contributes to progenitor expansion. A major step in mRNA translation controlled by eIF4F is polysome recruitment. Aims. Our objective is to identify genes that are translationally controlled upon SCF signalling and to investigate their contribution in the attenuation of erythroid differentiation. Methods. To identify genes whose expression is regulated by signalling-induced polysome recruitment, we compared total and polysome-bound mRNA from factor deprived (no SCF) and SCF-stimulated (20 ng/mL) Ba/F3 cells. Using micro-arrays, profiling was performed with 4 biological replicates and candidate genes were selected using ANOVA. In subsequent cluster analysis we combined these data with (polysomal) expression profiles of differentiating erythroid cells. Real time PCR was used to investigate if polysome recruitment of candidate transcripts are dependent on PI3K activation and eIF4E expression. Retroviral transduction was used to constitutively express these genes in the erythroid cell model and cell number, cell size and hemoglobinisation was measured to assess the effect on erythroid differentiation. Western blot analysis was used to investigate the levels of constitutively active IGBP1 on the phosphorylation status of mTOR targets. Results. We identified genes, upregulated specifically at the level of mRNA polysome recruitment and downregulated during erythroid differentiation. We further characterized 13 genes whose polysome recruitment is dependent on the PI3K/mTOR pathway. Constitutive expression of these targets in erythroid progenitors revealed that IGBP1 (Immunoglobulin binding protein 1) was able to inhibit erythroid differentiation. We elucidated a mechanism by which the IGBP1/PP2A complex prolongs the phosphorylation of mTOR targets, possibly the mechanism inhibiting erythroid progenitor differentiation. Summary/Conclusions. We identified in this study a novel and unique set of genes that are minimally regulated at the level of transcription and are translationally controlled upon SCF signalling. We support the importance of translation control to regulate the balance between expansion and differentiation of erythroid progenitors, by showing that IGBP1, a translationally controlled gene, blocks erythroid differentiation and this can be explained by maintenance of mTOR target phosphorylation under differentiation conditions. This suggests that, like SCF signalling and eIF4E overexpression, IGBP1 enhance translation initiation efficiency.
ONCOGENIC CBL MUTANTS CONFER A TRANSFORMING POTENTIAL TO HEMATOPOIETIC CELLS EXPRESSING THE FLT3 TYROSINE KINASE

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Background. CBL is a E3-ubiquitin ligase, that negatively regulates many receptor tyrosine kinases (RTK). The CBL gene is located on the human chromosome 11q23. Balanced translocations (t(4;11); t(11;14) and interstitial deletions involving CBL and a CBL-MLL fusiongene were described in patients with B-cell lymphomas and acute leukemias. Two oncogenic CBL deletion mutants, CBL-70Z and v-CBL, were isolated from murine retroviruses inducing B-cell lymphomas and acute leukemias in mice. The Fms-like tyrosine kinase 3 (FLT3) is expressed by the leukemic cells of 70-90% of patients with acute myeloid leukemia (AML) and acute lymphoid leukemia (ALL) and can contribute to malignant transformation by harbouring activating mutations, aberrant autocrine stimulation or overexpression. Aim. We hypothesised that CBL could play a role in regulating FLT3 RTK activity and, if mutated, could lead to aberrant FLT3 signalling in acute leukemias. Methods. CBL-WT and the CBL mutants (v-CBL, CBL70Z) were stably expressed alone or with FLT3-WT (FLT3-WT/CBL-WT, FLT3-WT/CBL-70Z and FLT3-WT/v-CBL cells) in murine IL-3 dependent Ba/F3 cells. Proliferation assays in the absence of IL-3, presence of FLT3 ligand (FL) and selective FLT3 inhibitors were performed. FLT3 activation status and the activation of downstream signaling pathways were investigated by western blotting. Results. Stable coexpression of FLT3-WT and CBL-70Z or v-CBL, but not FLT3-WT with CBL-WT or one construct alone, conferred IL-3 independent and FL-stimulated growth of FLT3-WTCBL-WT, FLT3-WTCBL-70Z and FLT3-WT/v-CBL expressing cells, but not of FLT3-WT/CBL-WT expressing cells. To determine whether the proliferative advantage of FLT3-WT/CBL-70Z and FLT3-WT/v-CBL cells is mediated by FLT3 we cultivated the cells in presence of selective FLT3 inhibitors, SU5614 and PCK412. Both inhibitors were able to abrogate the IL-3 independent and FL-stimulated growth of FLT3-WT/CBL-70Z and FLT3-WT/v-CBL cells. The FLT3-WT receptors were constitutively activated and showed a higher spontaneous dimerization rate in FLT3-WT/CBL-70Z and FLT3-WT/v-CBL expressing cells in the absence of FL. Analysis of the three important downstream signaling pathways of FLT3 (STAT5-, PISK/AKT- and MAPK-pathway) could show activation of STAT5 and AKT in FLT3-WT/CBL-70Z and FLT3-WT/v-CBL expressing cells. Summary. The CBL deletion mutants, CBL-70Z and v-CBL, but not CBL-WT, confer a transforming potential to hematopoietic cells expressing FLT3. The pro-proliferative phenotype of FLT3-WT/CBL-70Z and FLT3-WT/v-CBL cells is mediated by an increase in FLT3 tyrosine kinase activity compared to FLT3-WT/CBL-WT cells and can be inhibited by selective FLT3 inhibitors. Here, we provide a new mechanism of transformation mediated by FLT3: mutations of a regulatory protein, that is implicated in negative regulation of RTK kinase activity.


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Background. The role of myeloablative allogeneic stem cell transplantation (alloSCT) in the treatment of multiple myeloma (MM) is still an area of controversy. Few series have described the results of such a therapy as part of first-line treatment in younger patients. Methods. From 1985 to 2003, 116 patients with de novo MM less than 55 years were treated by alloSCT within 12 months following diagnosis either in first CR (n = 15), first PR (n = 70), stable disease (n = 14) or primary refractory (n = 19), and registered in the SFDM-TG database. Results. Patients were 67 males, 49 females, median age 41 years (18-55). The donor was a geno-identical one in 105 cases, and pheno-identical in 11 cases. The conditioning regimen consisted of 12 Gy total body irradiation (TBI) + 120 mg/kg Cyclophosphamide in 50% of the cases, TBI + Cy + melphalan in 20% of the cases, and TBI + melphalan in 17% of the cases. T-cell depletion was used in only 8 cases, and GVHD prophylaxis consisted of the combination of cyclosporine A + short course methotrexate in 85% of the cases. Grade 3-4 acute GVHD was documented in 18.7% and chronic GVHD in 30.5% of the cases, respectively. 100 days after alloSCT, the mortality rate was 28%, and 40% 1 year after alloSCT. The overall survival was 41% at 4 years, and 31% at 12 years, and disease-free survival was 26% at 12 years with a true plateau observed 5 years after alloSCT. Chemosensitive disease at the time of alloSCT and occurrence of chronic GVHD were associated with a better survival (12-year survival 38.2 vs 18.2% p=0.02, and 42 vs 20.1% p=0.002, respectively). Conclusion. With a prolonged follow-up, data from the SFDM-TG show that alloSCT when performed as part of first-line therapy, despite a high initial mortality, may cure one quarter of the patients with de novo MM less than 55 years.

NEW CRITERIA TO IDENTIFY RISK OF PROGRESSION IN SMOLDERING MULTIPLE MYELOMA: MULTIPARAMETRIC FLOW CYTOMETRY ANALYSIS OF BONE MARROW PLASMA CELLS

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Background. Smoldering multiple myeloma (SMM) is a monoclonal disorder defined by the presence of a serum monoclonal protein ≥3g/dl or bone marrow plasma cells ≥10% and absence of end-organ damage. These patients require close follow-up because the high risk of progression to symptomatic multiple myeloma. Therefore, the definition of new parameters that could be used for the identification of patients at the highest risk of progression could be of great importance in the clinical setting. Aims. To evaluate the impact of immunophenotypical analysis of bone marrow (BM) plasma cell (PC) for the prediction of risk of progression of SMM. Methods. From January 1996 to September 2004, bone marrow aspirate samples from 78 patients, who fulfil the criteria of SMM according to the International Myeloma Working Group criteria, were analysed by multiparametric flow cytometry. Plasma cells were immunophenotypically classified as normal or abnormal according to the expression of CD38, CD45, CD19 and CD56 antigens. Other parameters included were: percentage of plasma cell infiltration by morphology and cytometry, MC, immunoparesis, presence of Bence-Jones pro-
teurinia, haemoglobin, platelets, calcium, and albumin. The median age of the series was 69 years (range 45-88). The monoclonal paraprotein was IgG in 55 (65%), and IgA in 29 (34%), with a median paraprotein level of 2.5 mg/dL. The median follow-up was 50 months. Results. Thirty-six patients (42%) progressed to MM, with a median time to progression (TTP) of 26 months (range 2 to 94 months). Interestingly, flow cytometry showed that the number of aberrant PC (aPC) within the total PC (TPC) population in BM, clearly predict the risk of progression. Thus, in patients with ≥95% aPC from TPC the median TTP was 40 months vs not reached in the rest (p=0.0000). Other parameters with a significant influence on progression in the univariate analysis were: the paraprotein level (higher vs lower 3 mg/dL, p=0.0017), the presence of immunoparesis (no paresis vs. one or two Ig, p=0.0001), the presence of Bence-Jones proteinuria (p=0.0017), the total BM infiltration assessed both by morphology and flow cytometry (p=0.0324; and p=0.0001, respectively). Moreover, this cut off level of 95% aPC within TPC, also allowed us to discriminate two risk categories upon considering only patients at low risk of progression, based on a low paraprotein level or absence of immunoparesis (p=0.0072 and p= 0.0056, respectively). By multivariate analysis this new parameter (95% aPC from TPC), together with immunoparesis (no vs one or two Ig), BM infiltration by cytometry, and the amount of MC, had independent significant impact on risk of progression. Conclusion: bone marrow immunophenotypic analysis of plasma cells by multiparametric flow cytometry at diagnosis is useful for predicting progression of SMM into active MM.

A MULTICENTER, RANDOMIZED TRIAL OF ZOLEDRONATE VS OBSERVATION IN EARLY-STAGE, ASYMPTOMATIC MYELOMA

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Background. The use of bisphosphonates in patients with asymptomatic, otherwise untreated early-stage myeloma is still debated and currently not recommended by evidence-based guidelines due to a substantial lack of appropriate studies. Zoledronate is a third generation bisphosphonate, which significantly decreases skeletal events in active myeloma and has been demonstrated to have a possible anti-myeloma in vitro and in vivo effect. Aims. The aim of this study was to evaluate whether the prophylactic use of zoledronate is able to reduce the rate of and the time to evolution in overt, symptomatic myeloma requiring chemotherapy in this population of patients. Methods. On June, 2001, ten Italian centers started a randomized clinical trial comparing administration of zoledronate vs simple observation in patients with monoclonal gammopathy fulfilling the diagnostic criteria of asymptomatic, stage IA, IIA or smouldering myeloma, not requiring chemo-radiotherapy. Patients strictly diagnosed as having true MC were excluded. One-hundred sixty patients were enrolled and randomized (1:1) to receive (n 80) or not (n 80) zoledronate (Zometa, Novartis Pharmaceuticals, Origgio, Italy) for one year, on an out-patient basis, at the dose of 4 mg as 15‘ i.v. single monthly infusion. Results. No severe adverse events were recorded throughout the study, with the exception of a single patient treated with zoledronate who developed a reversible picture of osteonecrosis of the jaw. In the observational arm, six patients were lost at follow-up after 6-20 months. Asymptomatic hypocalcemia, without need of interrupting the treatment and promptly corrected by substitution therapy, occurred in fifteen of zoledronate-treated patients. Fever developed in seven patients receiving zoledronate, one of whom stopped the treatment after 3 infusions. Overall, no significant reduction of M-component (> 25%) was observed. On intention-to-treat analysis, after a median follow-up of 42 months, there were 19 (25.7%) progressions requiring treatment in the zoledronate group and 24 (30%) within the controls (p n.s.). Median time-to-progression was 19 and 16 months, respectively (p n.s.). Among the 36 patients who required chemo-radiotherapy in both arms, bone lesions and/or hypercalcaemia at the time of progression were observed in 16/20 (80%) of controls, and in 7/16 (43.7%) of zoledronate-treated patients (p=0.001). Conclusions. Our data indicate that the use of zoledronate in patients with early-stage, asymptomatic myeloma reduce the possibility of developing skeletal events at progression. Although a weak trend in favour of zoledronate arm was observed, in this study there was no statistical evidence about the possibility that zoledronate may also decrease the number of and/or the time to progression of the disease.

ARRAY-CGH DETECTS FREQUENT RECURRENT IMBALANCES IN PLASMA CELL DISORDERS


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Background and Aims. Genomic imbalances such as losses and gains occur frequently in hematological cancers. Their characterization in plasma cell disorders (PCD) is largely incomplete and few lesions (mostly identified by conventional cytogenetics and FISH) have been extensively studied for their pathogenetic and prognostic role. There is thus a clear need for a genome-wide screening of cytogenetically-cryptic lesions, through sensitive, robust and reproducible approaches. Array-CGH allows obtaining a comprehensive view of genomic imbalances, with a precise mapping of these aberrations to the genomic sequence. It has proven extremely successful in several diseases including chronic lymphocytic leukemia (CLL) which closely resemble multiple myeloma (MM) in terms of clinical and molecular heterogeneity. We have screened a panel of patients with PCD with the following Aims. 1) to identify the most common, yet undescribed genetic lesions; 2) to confirm these lesions by FISH; 3) to link genomic imbalances and clinical outcome. Methods. CD-138 purified plasma cells were employed. Genomic DNAs, from both the tumor and normal reference cells, labeled with different fluorescent dyes were cohybridized to 1 Mb resolution arrays (Spectral Genomics Inc. USA) containing 2600 Bacteria Artificial Chromosome (BAC) clones, according to manufacturers’ recommendations. Variations in DNA sequence copy number for each BAC clone was assessed by relative fluorescence signal intensities, providing a locus-by-locus measure of DNA copy-number changes. FISH experiments have been performed to confirm clonal abnormalities (gains/losses) identified by array-CGH. BAC clones were labeled for FISH experiments and interphase nuclei were made, according to standard cytogenetic protocols. Results. 20 patients were studied including 16 MM and 4 PCL. The median number of lesions/patient observed in our panel was 17 (range 4-135). The amount of the genome affected by chromosomal imbalances was highly variable among different patients (median 3.9% range: 0.14%-27%). This number is superior to that reported in CLL (Drandi, ASH 2005) and to a lesser extent to diffuse large B cell lymphoma (DLBCL) (Chaganti, Blood 2005). Of 2600 BACs 934 were always spared from genetic damage, 864 were targeted only in one patient, 401 in two patients, 296 in 3-5 patients and 105 were targeted in six patients or more. By restricting the analysis to the most common lesions we have identified five previously undescribed recurrent lesions occurring in at least 30% of patients, involving the following regions: 19p13.2, 14q12, 16q12.1 11q24, 9q23. We have confirmed the first two lesions by FISH, while for the others experiments are ongoing. Conclusions. 1) In PCD the genome undergoes a high degree of genetic disruption compared to other lymphoid tumors, particularly CLL; 2) a number of highly recurrent lesions have been identified and some have already been confirmed by FISH. All these lesions will require further investigation to identify candidate target genes and to verify if they might be prognostically relevant.
Hyperdiploidy and hypodiploidy in monoclonal gammopathy of undetermined significance and multiple myeloma, and their relationships with monosomy 13

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Background. Cytogenetic performed in multiple myeloma (MM) allow the definition of two pathways for malignant progression, one hyperdiploid and the other hypodiploid. Monoclonal gammopathy of undetermined significance (MGUS) is probably a preliminary step to MM for some patients at least. Cytogenetic status in MGUS is limited to interphase FISH techniques, due to the small amount of abnormal plasma cells and to their low proliferation rate. Aims. To define incidence of hyperdiploidy and hypodiploidy in MM and MGUS, and the distribution of monosomy 13 within each group. Methods: We ascertained DNA content of plasma cells (ploidy) using Feulgen staining and image analysis in 96 MGUS and 169 MM patients. Interphase FISH was performed using centromeric probes to look for trisomies 3, 7, 9, 11 and 15 in 42 MGUS and using rb-1 gene probe for monosomy 13 in 57 MGUS and 150 MM patients. Results. Hyperdiploidy and hypodiploidy were found in 54% and 11.5%, and in 59.5% and 25% of MGUS and MM patients respectively. Mean and median ploidies, for hyperdiploid as for hypodiploid, were similar in MGUS and MM. Interphase FISH confirmed the association between trisomies for several odd chromosomes and hyperdiploidy. Monosomy 13 was found in 24.6% in MGUS and in 45.3% in MM; incidence was similar in hyperdiploid MGUS and hypodiploid MM (38% and 31.9% respectively), whereas it was found in 11.1% of hypodiploid MGUS contrasting with 76.3% found in hypodiploid MM. Only two patients, both hypodiploid, evolved to MM after a mean follow-up of 77 months. Conclusions. Our results show that the number of hyperdiploid patients and the amount of chromosomes gained are similar in MGUS and in MM; monosomy 13 was found in equal numbers in both disorders, hypothesizing that events unrelated to monosomy 13 need to occur for evolution of MGUS to MM. In contrast, hypodiploidy is rare in MGUS, and is unrelated to monosomy 13, hypothesizing that hypodiploid MM might occur either after a MGUS step with deletion 13 as a secondary event, or using another pathway without prior MGUS.

HSV-TK donor lymphocytes add-backs enable a wider use of haploidential allogeneic transplantation by reducing infection related mortality and improving survival in high risk acute leukemia

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Background. Allogeneic transplantation from haploidential family donors (haplo-SCT) represents the ideal solution to offer to every and each patient with high risk leukemia the potential cure of allogeneic adoptive immunotherapy. However, the delayed immune reconstitution secondary to the required profound T-cell depletion compromise haplo-SCT with a high rate of late mortality and relapse. Methods. In a phase II multicenter trial (MM TK007), we explored early add-backs of donor lymphocytes genetically engineered to express the herpes simplex thymidine kinase (TK-DLI) suicide gene after haplo-SCT, in inducing early immune reconstitution and selective control of GVHD. Results. Thirty-one advanced age pts (median age 51, 17-64) were transplanted for high risk leukemia. No immune reconstitution and no graft versus host disease (GVHD) were observed in absence of TK-DLI. 17 pts received TK-DLI at a median dose of 107/kg with 1st infusion at d +42; 14 pts obtained a prompt and sustained immune reconstitution with CD3+ >100/mcl at a median time of 86 d (57-127) from SCT and 21 d (13-42) from TK-DLI. Six pts developed acute GVHD, (grade I to IV) that was always completely abrogated by ganciclovir. In patients in remission of leukemia at time of SCT, who were alive at day +42 and received TK-DLI, overall survival was 85% at 2 years (intention-to-treat analysis: 46%). The cumulative incidence of TRM and relapse showed a 40% probability of mortality with a median time of death of 90 days and last event at day +166. The cumulative infectious mortality beyond day 100 post transplant was 12.5% in our population, versus 53% of his-tomed patients at day +42; 14 pts received TK-DLI add-backs at a median time of 86 d (57-127) from SCT and 21 d (13-42) from TK-DLI. Six pts developed acute GVHD, (grade I to IV) that was always completely abrogated by ganciclovir. In patients in remission of leukemia at time of SCT, who were alive at day +42 and received TK-DLI, overall survival was 85% at 2 years (intention-to-treat analysis: 46%). The cumulative incidence of TRM and relapse showed a 40% probability of mortality with a median time of death of 90 days and last event at day +166. The cumulative infectious mortality beyond day 100 post transplant was 12.5% in our population, versus 53% of historical data. These data correlate with rapid development of normalization of the T cell repertoire documented by spectratype, followed by detection of high frequencies of T cells specific for CMV and EBV by gFNF ELISpot. Conclusions. These results indicate that TK-DLI abolish late mortality after CD34+ haplo-SCT in adults. Overall survival rates in patients treated with TK-DLI were superior as compared to survivals from large populations of haploidential SCT of EBMT registry database. A phase III randomized multicentric study will start in 2006 to validate prospectively the advantage of TK-DLI in haplo-SCT.

Differential immunomodulation of engraftment following MHC-mismatched hematopoietic stem cell transplantation by cotransplantation of host versus donor mesenchymal stem cells

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Background. Mesenchymal stem cells (MSCs) are multipotent progenitor cells that have emerged as a promising tool for clinical application. Further clinical interest has been raised by the observation that MSCs are immunoprivileged, and display immunosuppressive capacities. These properties might be of therapeutic value in allogeneic transplantation to prevent graft rejection and for the prevention and treatment of graft-versus-host disease. Aims. In the present study, we examined the in vivo immunomodulatory properties of MSC in murine models of allogeneic bone marrow (BM) transplantation. Methods. Balb/c or C57Bl/6 recipients were sublethally irradiated and transplanted with T-cell depleted bone marrow cells (Bone marrow cells (Bone marrow cells of major MHC mismatched) or Balb/b (multiple minor histocompatibility antigen mismatched) donors, respectively, with or without host, donor or third party MSCs. MSCs were expanded from host, donor or third party BM cells in medium containing FCS. MSCs were used in cotransplantation experiment at passage 7. Chimerism was assessed at various time points after transplantation by flow cytometry. Results. The cotransplantation of host-derived MSCs resulted in a significantly increased engraftment rate both in multiple minor mismatched recipients (82% versus 44% in the...
absence of MSCs) as in major MHC mismatched recipients (50% engraftment versus 17% in the absence of MSCs). Furthermore, the MSC-facilitated engraftment was still evident at 4 months after transplantation and the donor chimerism included both lymphoid (CD3+, B220+) and myeloid (GR-1+) lineages. In contrast, infusion of donor-derived MSCs was associated with a significantly increased rejection rate of allogeneic donor BM cells in both multiple minor antigen mismatched transplants (44% versus 0% in the presence of donor MSCs) and major MHC mismatched transplants (80% versus 22% in the presence of donor MSCs). Finally, the addition of third party MSCs derived from C3H mice did not affect the engraftment rate of MHC mismatched transplants. In vivo cytotoxicity data, employing differentially CFSE labeled splenocytes, showed that the infusion of merely allogeneic donor or third party MSCs in naive mice was sufficient to induce a memory T cell response. Summary/Conclusions. Taken together, these results show that MSCs are capable of modulating immune responses in vivo and that these responses are affected by MHC antigen matching between donors and recipients. In addition, MSCs are not intrinsically immunoprivileged and are capable of inducing a memory T cell response following injection in vivo in immunocompetent hosts. Following cotransplantation in immunocompromised hosts that have received sublethal irradiation, allogeneic MSCs still induce an allogeneic response resulting in graft rejection. Although it is still unclear whether the immunogenicity of allogeneic MSCs is preserved after a full myeloablative conditioning regimen, the possibility that allogeneic or third party MSCs are immunogenic may be taken into account in designing clinical studies in the setting of allogeneic stem cell transplantation.

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SUSTAINED RECONSTITUTION OF NADPH-OXIDASE ACTIVITY IN X-LINKED CHRONIC GRANULOMATOUS DISEASE FOLLOWING RETROVIRAL GENE THERAPY AND NONMYELOABLATIVE CONDITIONING

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Gene transfer into hematopoietic stem cells has been successfully used to correct immunodeficiencies affecting the lymphoid compartment. However, similar results have not been reported for diseases affecting myeloid cells, mainly due to low engraftment levels of gene-modified cells observed in unconditioned patients. Here we report on two adult patients who received gene-transduced hematopoietic stem cells in combination with nonmyeloablative bone marrow conditioning for the treatment of X-linked Chronic Granulomatous Disease (X-CGD), a primary immunodeficiency caused by a defect in the oxidative antimicrobial activity of phagocytes. Therapeutically significant gene marking was detected in neutrophils of both patients leading to large numbers (up to 60%) of functionally corrected phagocytes 16 months after gene therapy. This high correction resulted from an unexpected but temporarily restricted expansion of gene transduced myeloid cells in vivo. Gene marking levels in B-cells has remained constant at a level of 20%, while gene marking in T-cells is below 5%. Gene marking in bone marrow was detected at levels between 30% and 40% one year after transplantation. Killing assays in vitro have demonstrated antibacterial and antifungal activity in gene transduced phagocytes and both patients recovered of Staph. aureus and A. fumigatus infections after gene therapy. Both patients have been free of severe bacterial and fungal infections since transplantation. Large-scale mapping of retroviral integration site distribution revealed that activating insertions in the zinc finger transcription factor homologs MDS1/EVI1, PRDM16, or in SETBP1 have expanded gene-corrected long term myelopoiesis 3- to 4-fold in both patients, providing direct evidence in humans that these genes influence regulation of normal long-term hematopoiesis. Although it is likely that insertional effects still reinforced the therapeutic success observed in this trial, our results suggest that gene therapy in combination with bone marrow conditioning is a therapeutic option for inherited diseases affecting the myeloid compartment and can be successfully used to treat CGD.

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IDENTIFICATION OF THE VON WILLEBRAND FACTOR BINDING SITE IN COLLAGEN USING TRIPLE HELICAL PEPTIDES

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Background. The interaction of the plasma protein von Willebrand factor (VWF) with subendothelial collagen initiates adhesion of blood platelets to the damaged vessel wall or ruptured atherosclerotic plaque. A detailed molecular description of the VWF-collagen interaction may facilitate development of a novel class of antithrombotic drugs that inhibits this vital step in platelet thrombus formation. Aims. We have previously used site-directed mutagenesis to map the collagen-binding site in the VWF A3 domain. Here, we aimed to identify the amino acid sequence in collagen type III which mediates VWF binding. Methods. We have synthesized a set of 57 peptides, each containing 27 amino acids of native collagen sequence flanked at each end by five GPP (standard amino acid nomenclature) triplets which support the triple helical structure that is essential for ligand recognition by collagen. The sequence of each peptide overlaps by 9 amino acids with that of each adjacent peptide. VWF binding to these peptides was assessed by a solid phase binding assay. Also, platelet deposition from whole blood under flow to peptides that interact with VWF was assessed. Finally, we used the information obtained from these binding experiments to construct a molecular model of the collagen-VWF interaction. Results. A single peptide from this set (#23) was shown to bind VWF in a solid-phase binding assay. The affinity of peptide #23 for VWF was comparable to that of native collagen type III. The peptide #23-VWF interaction was abolished by a monoclonal antibody directed against the collagen-binding site on the VWF A3 domain. Furthermore, recombinant VWF variants that were previously shown to lack collagen-binding capacity (delta A3, His1023Ala) were not able to bind to the peptide. Immobilized peptide #23 also supported platelet adhesion from whole blood under flow conditions. Platelet adhesion to peptide #23 could be abrogated by a monoclonal antibody directed against the VWF A3 domain, which inhibits the interaction of VWF with full-length collagen. We subsequently synthesized a set of truncated and alanine-modified triple helical peptides based on the sequence of #23, which were all tested for VWF and platelet binding from whole blood under flow conditions. Modified peptides either strongly interacted with both VWF and platelets, or lacked both VWF and platelet binding. Based on these experiments, we identified the sequence RGQGQWMGF (O is hydroxyproline) as the minimal VWF binding sequence in collagen type III. Mutation of either O or M to alanine (A) did not affect VWF binding, whereas replacement of R, O, V, and F by A completely abolished VWF binding. Glycine residues were not replaced, as they are essential for triple helix formation. A model of the VWF A3 domain with this nonapeptide collagen sequence was constructed and we have detailed insights into the VWF-collagen interaction. Conclusion. We have identified a 9 amino acid sequence in collagen type III that is entirely responsible for high affinity binding to VWF. The detailed molecular description of the VWF-collagen interaction described here may facilitate development of agents disrupting this interaction, which may have potential as antithrombotic drugs.
Our aim is to study the role of Pten in other self-renewing tissues such as decreased apoptosis and increased self-renewal (Groszer et al., 2001). Recently it was shown that conditional deletion of the Pten gene causes an increase in neuronal stem/progenitor cells due to mature organism. Deficiency of Pten in mice causes early embryonic lethality preventing the analysis of Pten function during development or in the mature organism. Recently it was shown that conditional deletion of the pten gene causes an increase in neuronal stem/progenitor cells due to decreased apoptosis and increased self-renewal (Groszer et al., 2001). Our aim is to study the role of Pten in other self-renewing tissues such as the hematopoietic stem cell compartment. We generated a mouse line in which the pten gene can be deleted in hematopoietic cells including hematopoietic stem cells (HSCs). This was achieved by crossing the conditional pten (ptenfloxed allele with the IFN-α inducible MxCre transgene. Cre activity was induced in 4 week old MxCre;ptenfloxed/fox allele mice, converting the ptenfloxed alleles into pten (delta) null alleles in HSCs and other hematopoietic cell types present in the bone marrow. Pten mutant mice show severely enlarged spleens, due to a dramatic expansion of granulocytes, erythrocytes and megakaryocytes. In addition, mutant mice accumulate immature cancer-like cell types and develop transplantable leukemias within 4-6 weeks. Furthermore, normal numbers of HSCs are present in the bone marrow, however the total number of phenotypic HSCs is 6-fold increased in the spleen. Label retaining assays indicate that the quiescent HSC pool in the bone marrow is lost in Pten mutants suggesting that the P13-kinase pathway controls the transition of HSCs from a long-term quiescent to a self-renewing mode. In summary these results suggest that Pten activity restricts HSC self-renewal, and that it functions as a tumor suppressor in the hematopoietic system.
Transfusion medicine

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BLOOD-SHOT EYES: AUTOLOGOUS SERUM EYE DROPS FOR GRAFT-VERSUS-HOST DISEASE AND OTHER DISORDERS

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Background. Autologous serum eye drops have been used for treatment of ocular surface disease including chronic corneal ulceration and severe dry eye caused by graft-versus-host disease (GVHD), Stevens Johnson syndrome and Sjögren’s syndrome. Autologous serum eye drops are hypothesized to be superior tear replacement to commercially available lubricants because (1) serum contains similar elements to natural tears, such as epidermal growth factor & vitamin A, facilitating corneal healing and providing a practical immuno-biologic barrier, (2) the autologous source limits antibody formation & allergic reactions, allowing longer term therapy. Aims. To provide safe, high quality autologous serum eye drops for treatment of patients with refractory corneal ulceration and dry eye syndromes. Methods. Collection, processing and testing of units occurs at the Australian Red Cross Blood Service (ARCBS) GMP-licensed facility. From 2000-2005, eye drops were sterilised vials at -30°C. When required, aliquots were thawed, diluted with 120 mL sterile 0.9% saline and transferred into sterilised 5mL eye drop bottles. All manipulations were performed in a class II biohazard cabinet using sterile consumables. Since September 2005 we use the following fully closed system: Whole blood donations (up to 450 mL ± 10%) are collected from patients meeting ARCBS autologous donor eligibility criteria. Collection bags without anticoagulant (Baxter trio dry packs) facilitate clotting and serum removal following centrifugation. Serum is diluted to 20% by adding 200 mL sterile 0.9% saline to each 50mL serum vial via sterile connection. A sample is taken for microbial testing, and the remaining volume transferred into 20 metres tubing of a Macopharma blood bag. Tubing is segmented at ~7 cm intervals, creating approximately 200 segments (individual doses) from a single donation. These are frozen at < -18°C. Labelled storage containers are stored in local hospital blood bank freezers and/or by patients at home. Patients use the drops according to clinical need in consultation with their doctor. One donation can provide supplies for approximately 6 months. Information sheets for patients, doctors and hospital laboratory staff reinforce appropriate product storage and handling. Results. We have collected blood from 52 patients. Clinical indications include GVHD, Sjögren’s syndrome/scleroderma, Stevens Johnson syndrome, and epithelial defects following corneal grafts. One patient was excluded (hepatitis B positive). No complications were reported. Some patients require therapy for short term indications, others have used the drops for extended periods, the longest being nearly 6 years. The 20% serum dilution, chosen in 2000 based on available literature, appears optimal to prevent eye crusting while maintaining adequate potency and a practical interval between transfers. The 20% serum dilution, chosen in 2000, was discarded for a positive microbial culture. No complications were reported. Some patients require therapy for short term indications, others have used the drops for extended periods, the longest being nearly 6 years. The 20% serum dilution, chosen in 2000 based on available literature, appears optimal to prevent eye crusting while maintaining adequate potency and a practical interval between transfers.

0537
ERYTHROID CRISIS CAUSED BY PARVOVIRUS B19 TRANSMITTED VIA RED BLOOD CELL TRANSFUSION - ITS DIRECT DETECTION BY PCR DIRECT SEQUENCING METHOD

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Background. The administration of blood products possesses certain risk of transmission of a variety of viruses including parvovirus B19. Parvovirus B19 (B19) is a small non enveloped erythrovirus that can cause various clinical manifestations. B19 infection has been reported to cause erythroid crisis in immunocompromised hosts including patients with AIDS, malignancies, or patients undergoing chemotherapy or organ transplantation. The virus is normally transmitted via respiratory tract; however, transmission via the administration of blood products has also been speculated. The infectivity of B19 in the blood products is affected by the level of anti-B19 IgG in the products as well as the recipient immune status. Therefore, it is often difficult to prove directly whether the B19 transmitted via blood transfusion does cause erythroid crisis. We here report a case of erythroid crisis after red blood cell (RBC) transfusion, in which we could successfully detect the same B19 virus as in the blood product. Patient. A 41-year-old Japanese man was admitted to our hospital for the treatment of hairy cell leukaemia in May 2005. He was treated with cladribine (0.09 mg/kg/day) for 7 days. 16 days after the treatment, hairy cells in the peripheral blood became undetectable, but the patient became anemic and received 2 units of RBC transfusion. He remained anemic with reticulocytopenia even after the recovery of granulocytes. The diagnosis of B19 associated with PrCA was made according to the presence of B19-specific IgM antibody and viral DNA in sera. To assess whether the B19 was transmitted via the blood product, we performed PCR direct sequencing analysis of B19 in the patient and his blood donor’s sera. The DNA sequence of 2 distinct regions of the genome in B19 virus (NS1 and VP1) were amplified and then directly sequenced. Sequencing of NS1 and VP1 regions from the patient was completely corresponded to those of blood donor serum. The results suggest that B19 virus is horizontally transmitted from the blood products, and it may be the cause of erythroid crisis in the patient. The patient was treated with intravenous immunoglobulin (5 g/body for 10 days) without any response. Erythropoiesis of the patient began to recover spontaneously around 50 days after the treatment, and B19 virus DNA became negative by PCR analysis. Conclusion. This is the first case report demonstrating the transmission of B19 via RBC transfusion did cause erythroid crisis in the recipient, by using genomic PCR direct sequencing method. Blood products containing B19 DNA may possess a potential risk, especially for immunocompromised patients. Therefore, more sensitive screening for detecting B19 virus should be applied especially for transfusion in these patients.

0538
USE OF TAGVHD WARNINGS IN PATIENTS RECEIVING PURINE ANALOGUE CHEMOTHERAPY

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Background. Transfusion associated graft versus host disease (TaGVHD) is a rare but serious complication of blood transfusion with mortality rates >90%. It occurs following the transfusion of immuno-competent donor lymphocytes. These engraft and proliferate in a susceptible host causing widespread tissue damage. TaGVHD can be prevented by γ irradiation of cellular blood components for patients at risk. A major risk group is patients who have received purine analogue chemotherapy, principally Fludarabine. The annual SHOT report demonstrates that incorrect component transfused remains the commonest transfusion error and many of these are accounted for by not selecting irradiated blood. Aims. To find if patients who had received Fludarabine had 1) a BCSH/NBS TaGVHD sticker in their notes 2) a flag on the hospital transfusion laboratory (HTL) IT system indicating the requirement for irradiated blood 3) a warning in the nursing profile regarding the requirement for irradiated blood. An additional aim was 4) to confirm correct carriage of results at all HTL sites. Methods. A total of 125 patients who had received Fludarabine chemotherapy were identified via pharmacy records. 2) Patient case records were traced and inspected for a BCSH/NBS TaGVHD sticker or other warning indicating the need for irradiated blood. 3) The HTL IT system was examined for a flag indicating the requirement for irradiated blood and checked to determine if blood subsequently transfused had been irradiated. 4) Nursing profiles for the patients were traced and examined for comments on the requirement for irradiated blood. Results. 1) Case records were obtained for 28/34 patients at site 1 and 14/14 patients at site 2. 2) At site 1 only 7/28 (25%) had any warning for the requirement for irradiated blood and only 2 (7%) had the BCSH/NBS TaGVHD sticker. At site 2 all 14 (100%) case records contained a warning regarding the requirement for irradiated blood, however, 0/14 had the BCSH/NBS sticker. 3) Only 1/34 patients (site 1) and 1/14 patients (site 2) did not have a flag on the HTL IT system regarding the requirement for irradiated blood and neither of

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these patients had been transfused. All cellular products transfused sub-
sequent to Fludarabine therapy had been irradiated. 4) Fludarabine
prescription.

Conclusions. 1) The high rate of warning flags in The HTL.
IT system indicates that medical and/or biomedical staff are aware of
the need for irradiated blood in these patients. 2) The BCSH/NBS
stickers are not always used. It would seem likely that patients are unaware
of this potential risk from transfusion. 3) Although transfusion within
the patient's institution may be safe, results indicate that transfusion at
another site is likely to be potentially hazardous. We suspect that these
findings are not unique to these 2 centres. The findings of this study have
led us to alter our practice with pharmacy staff distributing the
BCSH/NBS label, addition leaflet and sticker at the time of first
Fludarabine prescription.

0539
FACTORS DETERMINING THE RISK OF SEVERE (WHO GRADE 3 AND 4) HEMORRHAGE IN
HEMATOLOGIC PATIENTS
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Current indications for platelet transfusion in the management of
thrombocytopenia hinge on studies, that used minor bleeding as the
end point. Minor bleeding may not be a good correlate for life-threat-
ening hemorrhage, that poses real risk of death from that cause. There-
fore, we have attempted to verify these indications through evaluation
of severe hemorrhages. In this study, we have analyzed retrospectively
circumstances of 146 severe hemorrhages (20 grade 3 hemorrhages, and
126 grade 4, among them 109 fatal) that have occurred among 1590
patients hospitalized because of various hematologic disorders with a
goal to identify factors that might have contributed to the occurrence of
hemorrhage. It was found that unintentional violation of platelet trans-
fusion policy (no transfusion for patients with platelet count below
10x10^9/L) might have been responsible for 8 such hemorrhages, at the
most. Similarly, 8 hemorrhages have occurred in patients with normal
or increased platelet count. Frequency of remaining 130 hemorrhoses
was inversely correlated with platelet count. Tendency for increased
number of hemorrhages started with platelet count below 50x10^9/L,
become significant below 40x10^9/L, and further increased until below
20x10^9/L with plateau afterwards. The highest frequency of hemorrhag-
eses was in patients with various forms of acute leukemia, either primary
or secondary and aplastic anemia (16-80% of all patients with given
form of disease had severe hemorrhage) and was much lower in vari-
ous lymphomas (1 and 6%). Moreover, almost half of hemorrhages in
acute leukemia has occurred in patients with early disease (within 50 days of diagnosis). The lowest frequency of hemorrhages was for ITT, when only one hemorrhage among 72 patients has occurred. For patients with platelet count between 20 and 50x10^9/L concomitant presence of plasma clotting factor abnormalities was an important fac-
tor contributing to the occurrence of hemorrhage. Unexpectedly, the
presence of severe infection had no effect on hemorrhage occurrence in
that group. These data may suggest that in order to effectively prevent
life-threatening hemorrhaghes in patients with early acute leukemia it
may be necessary to increase transfusion threshold to at least 20x10^9/L
in this group of patients only. Moreover, the lower number of grade 3
than grade 4 hemorrhages in that cohort may suggest that there is a dis-
proportion between benign and severe hemorrhages in thrombocyto-
openic patients possibly related to the undefined differences in the
resistance of blood vessels in particular patients to the injury.

0540
LONG-TERM ERYTHRO-EXCHANGE IN THE TREATMENT AND PREVENTION OF SEVERE
SICKLE CELL DISEASE RELATED COMPLICATIONS IN SYCILY
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Background. Sickle Cell Disease (SCD) is a severe health problem in
Sicily. Among the Sicilian population some individuals have frequent
vaso-occlusive complications, whereas others have sporadic/mild
episodes of SCD. Although the incidence associated with SCD are numerous, both direct and indirect, essentially all are associat-
ed with recurrent vascular occlusions. If the goal of therapy is to increase
the capacity for oxygen transport and drastically reduce the levels of
HbS, the therapy should be erythro-exchange and not simple transfu-
sion. Erythro-exchange is indicated for the treatment of vari-
ous severe complications of SCD, such as acute pulmonary syndrome,
stroke, and acute syndromes involving multiple organs. In this study,
from November 1999 through December 2005, we investigated a select-
ed group of patients affected by SCD who developed one or more severe
SCD-related episodes. The aim was to evaluate if a long-term exchange
therapy could play a role in reducing/or reducing rates of SCD compli-
cations, and to compare this management program with exchange therapy disadvantages. Materials and Methods. We studied 9
patients affected by SCD, 6 males and 3 females with a mean age of 38.2
years (range 24-62 years); informed consent was obtained from all sub-
jects. All patients refused Hydroxyurea treatment for personal reasons.
Although some classifications included 4 patients with HbSS, 2 patients
with HbSβ+βthal, and 3 with HbSβ+βthal were treated with Fludarabine.
Two Out Of The 9 Patients Developed One Stroke Episode, Five
Acute Chest Syndrome, And Two Recurrent And Severe Vaso Occlusive
Episodes. Acute Episode Treatment: analgesic regimen, hydration, oxy-
gen. therapy, antibiotics associated with early erythro-exchange. A sec-
ond exchange was performed after 5-4 days to stabilize HbS levels
between 20% and 45%. Long-Term Care And Follow-Up: exchange
transfusion was applied therapeutically as a prophylactic regimen and
the goal of the protocol was to maintain an HbS of ≤55% and ≥35%
pre and post pheresis. Transfusion guidelines include the use of units
matched with an extended phenotype. Patients were monitored for allo-
immune reactions and diseases 30 days after each exchange. Exchange
was performed for the first six months and twice per year during follow-up (4-5 years). Red cell exchange was performed using
a COBE (Lake-wood CO) Spectra TM continuous flow system. Results. the mean pre and post haemo-
globin was 9.5±0.85 g/dL and 11.1±0.93 respectively; haematocrit
29.5±2.91% and 33.7±4.31%; ferritin levels were 2385.4 ng/mL pre and
2955 ng/mL post exchange. All patients had a dramatic clinical improve-
ment hemorrhage, that poses real risk of death from that cause. There-
thereof, we have attempted to verify these indications through evaluation
of severe hemorrhages. In this study, we have analyzed retrospectively
circumstances of 146 severe hemorrhages (20 grade 3 hemorrhages, and
126 grade 4, among them 109 fatal) that have occurred among 1590
patients hospitalized because of various hematologic disorders with a
goal to identify factors that might have contributed to the occurrence of
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acute leukemia has occurred in patients with early disease (within 50 days of diagnosis). The lowest frequency of hemorrhages was for ITT, when only one hemorrhage among 72 patients has occurred. For patients with platelet count between 20 and 50x10^9/L concomitant presence of plasma clotting factor abnormalities was an important fac-
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than grade 4 hemorrhages in that cohort may suggest that there is a dis-
proportion between benign and severe hemorrhages in thrombocyto-
openic patients possibly related to the undefined differences in the
resistance of blood vessels in particular patients to the injury.

0541
HAEMOLOGINANCE FOR THE TRANSFUSION THERAPY OF PATIENTS WITH THALASSAEMIA
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Background. Multi-transfused patients are exposed to an increased risk of developing alloimmunization and other immune or non-immune
mediated transfusion-associated adverse reactions as well as contracting
transfusion-transmitted infections. Aims. Against this background, haemovigilance systems offer useful information about current prac-
tices both in the laboratory and in the clinical setting of transfusion, as
well as a tool for evaluating quality management procedures and pro-
viding blood safety indices. In this way, quality improvement may be
monitored at national and local level, to the benefit of all patients in
need of transfusion as well as those whose life depends on regular trans-
fusion therapy. Methods. In Greece, an HbA1c monitoring system initiated in November 1995, we analyzed immune, non-immune and infectious adverse reactions and adverse events associated with the transfusion of red cell concentrates (RCCs) in patients with thalassaemia.
We examined numerator data, such as the absolute number of adverse
events/adverse reactions reported in 1997-2004 during or after trans-
fusion. These data were analyzed by the type of reaction, severity ,
imputability and morbidity. Apart from iron overload - identified as the
predominant complication of multi-transfusion with RCCs - we studied
the incidence of non-haemolytic febrile reaction (NHFR) in relation to
leukoreduction (pre-storage, bedside and laboratory post-storage), the
transfusion reaction rate (TRR) and the patients’ reaction rate (PRR).
Acute and delayed haemolytic reactions and the incidence of alloim-
munization and autoimmunization were also investigated and analyzed
in relation to red cell antigen-matching policy. Transfusion of incorrect
RCCs, TRALL, TA-GvHD, allergic and anaphylactic reactions, infectious
and other adverse reactions were also examined. The data were then analyzed in relation to the number of transfusions (units of RBCs). Results: NHFTR incidence was 0.87% of blood units. TRR was 0.87% and PRR 7.2%. Further analysis showed that the TRR was 0.2% and PRR 1.8% in patients transfused with pre-storage leukodepleted RCCs, compared to 0.6% and 5.2% respectively with bedside filtered RCCs. Delayed haemolytic reaction was diagnosed in 47 patients (64%) had one antibody, 25% two antibodies and 13% multiple antibodies. Alloimmunization was diagnosed in 3.5% of the patients. The most common antibodies detected were of the Rh system, K, Kpa, Leë, Fy, Fyu and S. Alloimmunization of the IgG type was diagnosed in 1.5%. One patient with S-thal died of hyperhaemolyis syndrome (DAT positive of IgG type, anti-C3 and anti-C5d present) in the year 2004. The seroprevalence of HbsAg was 1%, of anti-HCV 60%, of anti-HIV 1% and of anti-HTLV 1%. The residual risk for HIV in this group of patients is 1.150,000 units. Yersinia enterocolitica and Klebsiella have been reported in 13 and 2 patients, respectively, and Malanita falcarum in 2 patients. Conclusion: Alloimmunization and autoimmunization were low in relation to the number of blood transfusions, while hyperhaemolyis caused the death of one S-thal patient. TRR and PRR due to NHFTR were significantly lower in pre-storage leukodepleted blood compared to bedside filtration. These haemovigilance data show that further improvements are necessary in leukoreduction, RBC washing and phe-nocompatibility policies.

### Table 1. Haemovigilance data for thalassemic patients during 1997-2004.

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>Issued RBCs</th>
<th>Acute component</th>
<th>Infections</th>
<th>Delayed NHFTR</th>
<th>Allergic/Anaphylactic TRALI</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>133*</td>
<td>292,599*</td>
<td>9</td>
<td>0</td>
<td>67</td>
<td>588</td>
<td>521</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>17</td>
<td>134</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.0%</td>
<td>0.0%</td>
<td>1.3%</td>
<td>10.0%</td>
<td></td>
</tr>
</tbody>
</table>

*46% of total; **18.5% of total.

### 0543

**MEMBRANE AND CYTOSKELETON PROTEIN CARBONYLATION IN NON-LEUKODEPLETED CPDA-PRESERVED RED BLOOD CELLS**

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**Background.** Despite the arrest of the normal aging process, *ex vivo* storage causes a number of reversible and irreversible biochemical and chemical changes to the red blood cells (RBCs) and accumulation of bioactive substances in storage medium, collectively referred to as *storage lesion*. Some of the negative effects of RBC transfusion are associated with the storage lesion. The importance of RBC oxidative damage in storage lesion is not well documented. **Aims.** To determine the possible storage-induced membrane and cytoskeleton protein oxidation in CPDA-preserved non-leukodepleted RBCs in the course of transfusion period storage. **Methods.** RBC concentrates from six eligible blood donors were prepared according to the standard operating procedures. Each RBC unit was followed up during the storage period of 35 days and shortly afterwards. Membrane skeletons were prepared by Triton X-100 extraction of ghosts. The membrane ghosts and skeletons of days 0-2 of these units, in addition to fresh preparations from ten healthy subjects, were used as controls. Total ghosts and membrane skeletons were analyzed by SDS-PAGE densitometry and immunoblotted against a variety of erythroid-specific antibodies. Carboxylated protein content was determined following 2,4-dinitrophenylhydrazine derivatization and SDS-PAGE coupled with Western blotting. **Results.** Immunoblotting with dinitrophenol-specific antibody revealed increased RBC membrane and cytoskeleton protein carboxyls with prolonged storage in CPDA blood bags. A quantitative and statistical important difference in carboxyl content was detected in membrane and cytoskeleton proteins isolated from RBCs stored for different time periods. In comparison to control membranes, there was an evident increase in the number and the intensity of the carboxylated protein bands appearing in the immunos- tained gel, ranging from MW 240 kDa to 15 kDa. The membrane skeletons stored even for long times in CPDA did not exhibit signs of severe proteolysis, as confirmed by immunoblotting analysis of spectrin, actin and 41 kDa proteins. **Summary/Conclusions.** We conclude that the protein carboxylation of RBC membrane and cytoskeleton during banking in CPDA is increased probably in association with the diminution in total antioxidant activity of RBCs. Since the stored RBCs convey less glucose to the pentose phosphate pathway, due to the subsequent decrease in NADPH and ATP levels, there are expected to be better protected against oxidative stress. The specific carboxylation of a set of RBC membrane and cytoskeleton proteins with prolonged storage in CPDA is shown for the first time and supports the concept of protein oxidation as a part of storage lesion. These data could give additional, useful information in evaluating improved conditions for storage of RBCs intended for transfusion.

### 0544

**PLATELETS RECOVERY AND TRANSFUSION NEEDS AFTER REDUCED INTENSITY CONDITIONING ALLOGENEIC PERIPHERAL BLOOD STEM CELL TRANSPLANTATION**


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Few data are currently available regarding platelets transfusion needs and the kinetics and predictive factors for platelets recovery after RIC allo-SCT. In this study, we analyzed the profile of platelets recovery and transfusion needs in the first 100 days after sibling PBSC RIC in a single institution series of 166 consecutive transplantations. Patients and graft characteristics were: age 49 y. (range: 13-70), diagnoses: 66 myeloid malignancies (40%), 64 lymphoid malignancies (39%), and 36 metastatic solid tumors (21%). 112 pts (67%) received an ATG-based RIC, while 54 pts (33%) received a low dose irradiation-based RIC. 75 pts (45%) developed grade 2-4 acute GVHD. Platelets recovery (>20 G/L) was positively correlated with increased cell density and membrane rigidity that are also evident in RBC storage. These data corroborate the evidence for the occurrence of oxidative damage in membrane proteins of stored RBCs and suggest the possible use of antioxidants in the RBC stored units intended for transfusion. They partially address the pathophysiological mechanisms underlying the RBC storage lesion and add some new insight in the field of RBC storage as a cytoskeleton-associated pathology.
observed at a median of 9 days (range: 0-99). The kinetics profile of platelets recovery is shown in the figure below. In the whole study population, the nadir was observed around day +7 after allo-SCT, and a plateau was reached about day +35. Filtered and irradiated donor apheresis platelets were used and patients needed a median of 1 unit (range: 0-55). In this series, 83 pts (50%) did not require any platelets transfusion during the follow-up period (median follow-up: 442 days) and 85 patients (50%) received at least one transfusion of platelets (54 were not transfused beyond day +100 after allo-SCT). Platelets count prior to RIC allo-SCT (median count 144 G/L; HR 0.44 (0.28-0.7) p=0.002, conditioning regimen (use of ATG; HR 1.86 (1.08-2.7) p=0.025) and the occurrence of acute (HR 1.54 (1.27-2.0) p=0.001) and severe GVHD (HR 2.36 (1.38-3.8) p=0.0006; 52% of patients with grade 3-4 acute GVHD were transfused) were the parameters significantly associated with platelets transfusion needs in Multivariate analysis. In this cohort, 145 pts could be assessed for platelets recovery at day +100: among them, 99 (68%) had a platelet count >99 G/L. Univariate analysis found a significant impact of AGVHD (p<0.0001) and Platelet count prior conditioning (p=0.012) but only acute GVHD (HR 5.52 (2.68-12.25); p=0.025) was associated with a delayed platelet recovery in a multivariate model. No impacts of pathology, GVHD prophylaxis regimen or CD34+ cell dose were demonstrated. Overall, these observations show a significantly lower rate of platelets transfusions and a quicker kinetic of platelets recovery after RIC allo-SCT and point out the effect of acute GVHD. In addition, considering the low level of myeloablation observed, RIC could be an appropriated field of investigation for the testing of megakaryocyte stimulating agents, towards further improving the safety and outcome of RIC allo-SCT.

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**0545**

**CHANGES OF HAEMOSTASIS INDUCED BY LDL-APHERESIS**

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**Background.** Familial hypercholesterolaemia and familial combined hyperlipidaemia are genetic disorders, which are associated with high incidence of severe cardiovascular complications. Extracorporeal elimination is used for selective removal of LDL-cholesterol in severe hypercholesterolaemias as combined strength of conservative and invasive lipid-lowering therapy may reduce progression of atherosclerosis in these high-risk patients. Activity of haemostasis plays an important role in the development of atherosclerotic complications. **Aims.** We hypothesize that LDL-apheresis reduces total plasma cholesterol and partially improves impaired haemostasis too. **Methods.** Repeated LDL-apheresis procedure (treatment interval 17.5±1.6 days) based on immunoadsorption has been used to treat nine patients with primary hypercholesterolaemia. Primary device Cobe-Spectra (USA); secondary device ADA (Adsorption-desorption automat, Medicap, Germany) with adsorbers Lipopak (Pocar, Russia). To assess changes of lipid metabolism and haemostatic parameters we analyzed many markers - in this branch of our study we measured plasma concentration of thrombomodulin (Asserachrom Thrombomodulin), von Willebrand factor (STA LIAtest vWF), t-PA (Asserachrom t-PA), PAI-1 (Asserachrom PAI-1) and fibrinogen (Fibrin-Prest automate S). We compared plasma concentration of all the above items before and after LDL-apheresis. In long-term monitoring we compared plasma concentrations before LDL-apheresis. All results were evaluated as proportional differences with software Statistica 6.0 (StatSoft Inc., Tulsa, USA). **Results.** LDL-apheresis procedure induced a significant interrelated decrease of total plasma cholesterol, thrombomodulin (-29.1%), von Willebrand factor (-15.6%) and fibrinogen (-21.7%). We have found no significant changes of all the above-mentioned markers in long-term monitoring (the levels of markers were compared before procedures during the period of 300 days). **Summary/Conclusions.** Therapeutic LDL-apheresis is an invasive and effective method, which not only reduces total plasma cholesterol but also partially improves impaired haemostasis too. Supported by Grant: MeIRI2007/99/06.

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**0546**

**METABOLIC MARKERS AND FUNCTIONAL PARAMETERS OF PLATELET CONCENTRATES COLLECTED BY MULTICOMPONENT APHERESIS WITH TWO DIFFERENT CELL SEPARATORS**

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**Background.** In the recent years the demand for blood components is constantly increasing, while exclusion criteria for donors are strengthened. With multicompartment collection (MCC) we are able to produce several standardized components during one blood donation session. **Aims.** In the present study we investigated platelet (plt) function and metabolic parameters in double plt concentrates (PC) collected by MCC and additionally to a packed red blood cell (PRBC). **Methods.** 15 donors were randomly allocated to either the TRIMA Access (Gambro BCT) or the AMICUS (Baxter) device and vice versa in the second procedure following a time interval of at least two months. The separators were programmed to collect 6×10^11 plts (2 units) and one unit of PRBC. Sample collections and analyses were done on day (d) 0 (donation day), d2 and d7. We determined blood cell counts (Sysmex SE-9500, Mueller), metabolic markers (Omni, Roche), LDH (Dimension Xpand, Dade Behring) and visual plt quality (swirling effect). Activation of coagulation was performed by INTEG Assay on the RÖTEM Coagulation Analyzer (Fenntapharm GmbH). To assess specific function of plts in clot formation the assays were repeated by addition of abciximab (Reopro, Centocor B.V.) and cytochalasin D (Sigma Aldrich). Maximum clot firmness (MCF) and difference of maximum clot elasticity (MCE) were calculated. Assays of hypotonic shock response (HSR) were performed on the SPA 2000 (Gettico-log). **Results.** Plt yields on d0 were 2.79±0.25 and 2.70±0.54×10^11/unit (Trima-PC and Amicus-PC). Metabolic markers are shown in the Table.

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![Figure 1. Analysis of Platelets recovery](image-url)
groups only on d0: 59.58±12.31% (T-PC) and 36.66±17.13% (A-PC). HSR increased significantly from d0 to d2 for the group of A-PC (d2: 53.55±15.47) and decreased significantly from d2 to d7 in the T-PC (d2: 62.66±9.05%, d7: 51.96±12.40%). Swirling effect was observed over the entire time period for all products although 2 A-PC showed aggregates on d0. Conclusion. The mean plt yield on d0 of the T-PC and the A-PC showed that these products have comparable plt counts. LuminexÒ markers were well maintained in both groups, although pH, glucose and bicarbonate were partially significantly lower, lactate, potassium and LDH partially significantly higher in the A-PC than in the T-PC. Thrombelastography of the T-PC showed significantly better in vitro function parameters only on d2, so did the HSR results of T-PC on d0. If the partially improved in vitro metabolic and functional parameters of the T-PC are of in vitro relevance has to be evaluated in clinical trials.

**0547**

**IVIG AND RITUXIMAB ALLOWS SUCCESSFUL SOLID ORGAN TRANSPLANTATION IN PATIENTS WITH A POSITIVE CROSSMATCH AND DONOR SPECIFIC ANTI-HLA ANTIBODIES**

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**Background.** Intravenous immunoglobulin (IVIG) in high doses is used increasingly for immunomodulation of various disorders. An illustrative case is end-stage renal failure (ESRF) where until recently, transplantation programs, and may be extended to other solid organ donors. This reinforces the important role of IVIG and rituximab in modern renal transplantation programs, and may be extended to other solid organ transplant programs in which the haematologist may be involved.

**0548**

**TRANSFUSION ASSOCIATED GRAFT VERSUS HOST DISEASE IN FOUR IMMUNOCOMPETENT PATIENTS**

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We observed transfusion associated graft versus host disease (TA-GVHD) in four male patients (ages: 61, 52, 56 and 57 years). All of the four patients showed the typical clinical and laboratory findings of TA-GVHD, as follows: high fever, diarrhea, erythodermia, hepatitis and pancytopenia. Oral micosisis was also observed in three patients. Skin biopsies performed in all the patients and were compatible with GVHD microscopic findings. Bone marrow aspiration and biopsy were performed in three of the patients and revealed hypocellularity. There are some other clinical similarities between our patients. All of the patients showed donor specific anti-HLA antibodies. Skin, oral and gastrointestinal tract biopsies revealed dermatitis or suppurative enteritis. All the patients had abnormal liver function tests. Some patients had high C-reactive protein levels. All of the patients had high serum creatinine levels. One of the patients had chronic active hepatitis. The patients were treated with low dose of IVIG (0.1 g/kg) with or without rituximab (375 mg/m²) for treatment of TA-GVHD. The patients were followed up for 1 year after the diagnosis. All the patients showed complete remission. The patients were discharged from hospital after 1 month of hospitalization. The patients were followed up in regular intervals. The patients were doing well with regular follow up.

**0549**

**THE CELLULAR COMPONENTS INDUCED IMMUNOSUPPRESSION VIA REGULATORY T CELLS IN ALLOGENEIC TRANSFUSION**

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**Background.** Transfusion save a lot of patients by supply of blood components, although there are some tolerable adverse effects. However, some clinical data showed that transfusion might be induced high risk of post-operative infection and higher relapse or mortality rate in cancer patients. There is controversial relation for relation between transfusion and immune dysfunction. Aims. We investigated whether immune dysfunction might be induced after transfusion of cellular components. Methods. We used 5 weeks old male BALB/c (H-2d, recipient), female C57/He (H-2k, donor), and C57/BL (H-2b, third party). We obtained irradiated spleen cells (SP) from BALB/c or C57/BL, and injected to C57/He mice via tail vein with intraperitoneal IL-2 administration. Some mice received consequent injection with same condition for 2 days. After 24 hours, we collected blood and bone marrow (BM), thymus, and spleen. For more examples, we used IL-10 in supernants from mixture with control and B-CHSP. Also, there were markedly increased CD4+CD25+ cells in BM, SP, and thymus with no change of other immune markers after 24 hours. However, in 2-day treated cases, there were increased some adhesion molecules and co-stimulatory markers. In cytotoxicity, SP after transfusion did not have cytotoxic effects against YAC-1 cell. For analysis of immune cells, we analyzed cell surface markers for each sample. Also, we evaluated cytotoxic effects against A20, YAC-1 cell and B-CHSP. We observed profound decrease of cell proliferation and, in some ratio, specific for H-2 complex. Two day transfusion did not show inhibition of cells but proliferation. For inhibitory effects of transfusion, we performed MLR with mixture of control and B-CHSP. B-CHSP SP were induced inhibitory effects according to mixture ratio. The usage of IL-10 in supernants from mixture with control and B-CHSP SP showed profound decrease of cell proliferation and, in some ratio, specific for H-2 complex.
toxic effects against A20, YAC-1 cells. Conclusion. We suggested that cellular components in transfusion might be contributed some immune regulatory effects by CD4+CD25+ cells just after 24 hours. Therefore, we have to consider patients' immune state after transfusion, in view of immune function.

0550
IMPLEMENTATION OF HAEMOVIGILANCE SYSTEM FOCUSING IN DONORS COLLECTION
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Introduction. HAEMOVIGILANCE (HV) consists of detection, gathering and analysis of information regarding untoward and unexpected effects of blood transfusion. It covers and surveys all activities of blood transfusion chain from donors selection to recipients. The European Blood Directive 2002/89/EC requires a HV network in each Member State. In our country a national regulation has come into force related to the implementation of HV. Materials and Methods. Blood donation leaflets and medical questionnaire guidelines for donor deferral were elaborated. Personnel were instructed to inform the incidents related to blood collection using a standard preformatted form. Analysis of the data collected during 2005. Informatic System from Blood Bank was used to obtain statistical data from donations. Results. Global donations in our Blood Bank in 2005: 6844. Incidents related to donation: 72 (1.05%). Incidents in males 0.82% (44/5343), females 1.86% (28/1501). Moment of the incident: while donation 59.7% (43/72) and post-donation 54.16% (39/72). Type of incident: haematoma (2.77%), thrombophlebitis (1.39%), local infection (1.39%), neurological lesion (1.39%), nausea/vomiting (12.5%), clonic movements (4.17%), incontinence (1.39%), unconscious (30.5%), tetanic (1.39%), citrate reaction (1.39%), problems in venous access (4.17%), dizziness (51.9%). Room conditions: heat (38.9%), cold (1.39%), insufficiency (4.17%). Donors characteristic: low weight 6 (3.3%), previous reactions 4 (5.5%), anxiety 8 (11.1%), first time 16 (22.2%), occasional donors 7 (9.72%), common donors 39 (54.16%), auto-transfusion 9 (12.5%). Graduation: immediate signs without life-treating risk and complete resolution 71 (98%) and immediate signs with life-treating risk and complete resolution 7 (9.8%). Immediate signs with life-treating risk (1.38%). Imputability: possible 45 (55.5%), suggestive 45 (55.5%) and sure 64 (8.85%). Conclusions. Incidents related to donation are slightly higher in females. The most frequent event is the sickness related to donation, in most cases accompanied by signs without life-treating risk and complete resolution and with a sure relationship with donation.

Aplastic anemia

0551
A STUDY OF HLA AND KIR GENES IN PAROXYSMAL NOCTURNAL HAEMOGLOBINURIA PATIENTS
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Background. Paroxysmal Nocturnal Haemoglobinuria (PNH) is characterised by the occurrence of haemolytic anaemia, thrombophlebitis and cytopenia. The expansion of a stem cell bearing a somatic mutation in the phosphatidyl-inositol glycan-A (PIG-A) gene, which is involved in the biosynthesis of the glycosyl-phosphatidylinositol (GPI) anchor, characterises this very rare haematopoietic disorder. Murine KO models clearly indicate the inability of pig-a mutation to account alone for the clonal dominance of the GPI-defective clones and for the development of PNH. A number of data suggest the involvement of T-cell-dependent and NK-mediated mechanisms in the selection/expansion of the GPI-defective haematopoiesis in PNH patients. Moreover, the role of HLA and KIR genes in the regulation of adaptive and innate immune response has been established. Aims and Methods. In order to investigate the involvement of immune-dependent mechanisms in the pathogenesis of PNH we addressed the analysis of HLA and KIR gene distribution in 12 PNH patients and in 217 controls of the same ethnic origin by PCR-SSP typing. In addition, 15 patients affected by Aplastic Anaemia (AA), whose immune-mediated pathogenesis has been already demonstrated, were enrolled in the study. The statistical evaluation of data was performed by using Student’s t test and Fisher two tailed exact test. Results. Our preliminary results demonstrate a significant increase of the HLA haplotype B*14, Cw*08 in PNH patients compared to healthy controls (36.3% vs 3.3%; p<0.005) while a not significant increase of this haplotype was observed in our group of AA patients (13.3% vs 3.3%; p=0.07). In addition, an increase of DRB1*15 was found in PNH (45.4% vs 20.1%; p=0.053) but not in AA patients. KIR analysis showed a decreased expression of KIR-2DS3 (10% vs 32.7%; p=0.110) and an increased expression of 2DS2 (80% vs 52.1%; p=0.077) genes in PNH patients respect to controls. Conclusions. The critical involvement of HLA molecules in the regulation of the adaptive immune response and the relevance of KIR-repertoire for the functional effectiveness of NK and cytotoxic effectors have been largely recognised. In this context, our data support the hypothesis that complex immune-mediated mechanisms could underlie the dominance of the GPI-defective clones in PNH. The occurrence of ethnical differences as well as the number of patients enrolled in this study are expected to account for the apparent divergence with the increased frequency of DR2 observed in 21 Japanese and 16 American PNH patients.

0552
EXPRESSION PATTERNS OF GLYCOSYLPHOSPHATIDYLINOSITOL-ANCHORED PROTEINS (GPI-AP) THROUGHOUT THE DIFFERENT NORMAL BONE MARROW CELL MATURATION PATHWAYS: A FRAME OF REFERENCE FOR UNDERSTANDING PNH
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Introduction. Glycosylphosphatidylinositol-anchored proteins (GPI-AP) are a heterogeneous group of proteins deficiently expressed in patients with paroxysmal nocturnal hemoglobinuria (PNH). Despite the physiological and pathogenetic relevance of different GPI-AP in PNH patients, no study has been reported in which the exact patterns of expression of a large number of GPI-AP are quantitatively evaluated in normal bone marrow (BM) cells, classified according to their lineage and maturation stage. Aim. In the present study, we have quantitatively analyzed the expression of eleven different GPI-AP (CD14, CD16, CD24, CD48, CD52, CD55, CD59, CD66b, CD87, CD119 and CD157) during maturation of the neutrophil, monocytic, erythroid, lymphoid, basophil and plasmacytoid dendritic cell (pDC) lineages in normal BM as a frame of reference for the understanding of the abnormal patterns of expression of GPI-AP observed in the BM of PNH patients. Material and Methods. Ten normal BM samples from an iden-
tential number of healthy donors were analyzed by flow cytometry, using different 6-color stainings—depending on the specific cell lineage under study—to analyze the expression of the above referred GPI-AP.

Results. Our results show that expression of most GPI-AP varies during normal BM maturation, and different profiles were frequently observed depending on the cell lineage or the GPI-AP analyzed. Accordingly, the expression of CD55, CD59, CD109, CD14, CD87, CD157, and CD48 were observed during monocyctic maturation. Different levels of expression of CD55, CD59 and CD58 were detected during the erythroid maturation. Maturation into basophils was associated with a higher expression of CD55 and a lower reactivity for CD59, both on this lineage and pDC. Finally, changes in the expression of CD55, CD59, CD52, CD24 and CD48 were observed along the B-cell maturation, whereas CD59 remains stable.

Conclusion. Our study shows that the expression patterns of most GPI-AP vary along the different normal BM maturation pathways, and provides a detailed map GPI-AP expression during normal hematopoietic differentiation, which could serve as a basis for the identification and characterization of changes occurring in PNH.

**0553**

**PREGNANCY-INDUCED PURE RED-CELL APLASIA, A DISTINCT SYNDROME**

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Background. Pure red-cell aplasia (PRCA) is a rare hematologic disorder. Several conditions have been associated with the development of PRCA including malignancy, infections, thymoma, autoimmune disorders, and rarely pregnancy. Previously, we have described a patient who developed PRCA on three occasions, 2 triggered by pregnancy and 1 secondary to medroxyprogesterone. Here, we systematically review the information on all published cases of pregnancy-induced (P)-PRCA.

Aim. To characterize the syndrome of P-PRCA. Patient and Methods. Published cases of P-PRCA induced by pregnancy were identified through MEDLINE (1966-July 2005; search terms: pregnancy and red-cell aplasia, pure) and references from journal articles, books, and abstracts. We excluded patients who developed PRCA prior to pregnancy, or had other etiologies. This analysis focused on the patient characteristics; clinical aspects of PRCA; pregnancy features, infant characteristics, treatment and outcomes. Results. Ten patients with 15 P-PRCA episodes have been reported. Patient characteristics. Age ranged from 15 to 40 years. Gestational age at presentation varied from the first to the third trimester. No patient had other causes of PRCA. P-PRCA. Hemoglobin level at presentation ranged between 2.5 to 9 g/dL. Bone marrow hypoplasia was observed in 7 patients. The average duration of sympotms was 1.5 weeks. Infant microcytic hypochromic red cells were treated with corticosteroids. Time to recovery of hemoglobin to a normal level ranged from 2 to 12 weeks post-partum, but was not described in three reports. Pregnancies. Four pregnancies ended with delivery via a Caesarean section, 1 at 30 weeks and 3 between 36-40 weeks of gestation. Three via vaginal delivery and 4 authors did not list the mode of delivery, all at full term. Two women underwent artificial abortions as treatment for PRCA. Infants. Fetal outcome included healthy infants in 8 cases and demise in 5. The cause of infant death was prematurity in 5, including 2 secondary to artificial uterine rupture, and not specified full term still birth in 2. Infant blood values were normal in the 9 reported cases. Follow-up. Five subjects had subsequent pregnancies, 3 complicated by PRCA; 1 normal and 1 spontaneous abortion without PRCA. One woman developed PRCA secondary to the contraceptive medroxyprogesterone acetate 3 years after her first episode and 4 years before her second occurrence of P-PRCA. Conclusions. P-PRCA is a self limited syndrome with a high risk for relapse during subsequent pregnancies. It can be managed by blood transfusions. Progestins might cause PRCA in these women. Physicians should closely monitor women with a history of P-PRCA if they become pregnant again or receive hormones for contraception or other reasons.

References


were found to carry a homozygous five base pair deletion (657del5) in the 6th exon of this gene. Recently a Turkish patient with NBS has been reported and who was found to be homozygote for 657del5. Aim: We reported seven patients with NBS from three different families in Türkiye. Families of the three homozygotes, whom we identified, originated from a Central Anatolian city of Konya (2 families) and an Aegean city, Izmir. These families denied any relationship with Slavic populations. Methods: All probands in these families were phenotypically diagnosed as having NBS based on growth retardation, microcephaly, developmental delay and facial features in addition to lymphoreticular malignancies. Cytogenetic and immunological investigations also supported the diagnosis. Results: We identified three Turkish families in which probands were diagnosed as having NBS and found to be homoygote for 657del5. Evaluation of haplotypes created with help of three flanking microsatellite markers revealed that the 657del5 allele in three Turkish families had a single origin, which was identical with that found in the Slavic populations. Conclusion: This study demonstrated that NBS has not been very rarely diagnosed in Turkish population. Our detection of homozygotes in these unrelated families suggests that the origin of malignancy implies that NBS is still underdiagnosed in Türkiye. 657del5 mutation in Turks shows the same origin described in Slavs. This result suggests the presence of population admixture in modern Türkiye.

0556 BONE MARROW EXPRESSION PROFILE OF RAS, PS3, AND MDM2 GENES IN CYTOPENIC DISORDERS

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Ras, PS3 and Mdm2 genes are active regulators of cell growth, division, and death. These 3 genes form a cascade in which each gene is able to modify the transcription and expression of the other gene. In order to understand the mechanisms of cytopenias in different hematologic disorders, we investigated the expression of these genes in 16 patients with mono, bi or pancytopenia, excluding myelodysplastic syndromes (complementary data already published in Blood 2004 104: Abstract 3433). The expression of P21ras, PS3, and Mdm2 proteins was detected in bone marrow cytopen cytopen by APAAP (alkaline phosphatase anti-alkaline phosphatase procedure) using monoclonal antibodies Y13-219, PAb 1801 and 2A10, respectively. N-, K-, H-ras and p53 gene mutations were assessed by PCR-SSCP (polymerase chain reaction/single strand conformation polymorphism) to exclude the presence of mutations in these genes. The quantitative wild-type expression of p21ras, PS3 and mdm2 proteins in the cytopen (combination of number of cells and staining intensity) was compared to the values of expression of these proteins in 7 normal bone marrows. We found that patients with severe aplastic anemia (n=4), bone marrow hypoplasia (n=3), and toxic leucopenia (n=2) as diagnosis exhibited total loss of cytoplasmic expression or notorious hypexpression of these proteins. Two cases of connective tissue disease (CTD) (1 undifferentiated connective tissue disease with positive PANCA; 1 systemic lupus erythematosus) strongly overexpressed both P21ras and wild-type PS3, while both cases of megaloblastic anemia (MA) aberrantly overexpressed wt-PS3 protein. The other 3 patients with familiar leucopenia, hepatitis B thrombocytopenia and hypersplenism due to schistosomiasis presented normal values of P21Ras, PS3, and Mdm2 proteins. We observed that P21ras, PS3 and Mdm2 proteins are downregulated in benign hypoplastic disorders of the bone marrow. Conversely, PS3 and P21Ras proteins are upregulated in bone marrow disorders in which the cytopenia and the activation of these proteins are likely triggered by DNA lesions as well as subsequent apoptosis as part of the pathophysiology of the disease in CTD and MA. Hence, we suggest that P21Ras, PS3, and Mdm2 genes are tightly and actively modulated in benign cytopenic disorders of the bone marrow and play a pivotal role in the molecular mechanism of these diseases.

0557 REDUCED INSULIN SECRETION IN NORMOGLYCEMIC PATIENTS WITH β-THALASSAEMIA MAJOR

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Background. Diabetes mellitus in patients with thalassaemia major is caused by hemosiderosis due to transfusional iron overload. However the exact mechanisms responsible for the progression from normoglycaemia to overt diabetes in these patients are still poorly understood. Aims: To assess insulin sensitivity and secretion in a fasting state in regularly transfused patients with β-thalassaemia major with normal glucose response during oral glucose tolerance test and estimate its possible relation to iron overload. Methods. We assessed fasting glucose, insulin and C-peptide levels from 24 patients with β-thalassaemia major and 18 healthy age- and body mass index-matched controls. Insulin sensitivity and insulin release index were calculated according to the HOMA model. The correlation to age, body mass index and serum ferritin was further analyzed. Results: Fasting glucose levels of patients were increased compared to controls (5.50±0.12 mmol/L vs. 4.67±0.13, mean±SEM, p<0.001). A decrease in β-cell secretion in the fasting state (estimated by SCHOMA) was observed in thalassaemic patients (SCHOMA 88.47±11.11 vs. 184.29±25.72 in controls, p<0.001). Further intragroup analysis of patients with impaired (IFG) and normal (NFG) fasting glycaemia group, revealed an increased SCHOMA in NFG compared to IFG patients (110.63±17.63 vs. 66.31±10.88 respectively, p<0.05) but no difference was found regarding estimated insulin sensitivity (ISI/HOMA) between the two groups. Plasma values of C-peptide correlated positively with ferritin (r=0.42, p<0.04) and SCHOMA (r=0.45, p=0.02) and negatively with ISI/HOMA (r=0.43, p<0.05). Conclusions. These results support the concept that an impairment of β-cell function, as reflected by a decreased insulin secretion index, already exists in β-thalassaemic patients with normoglycaemia before any changes in glucose tolerance can be detected in oral glucose tolerance tests.

Anemia / Red blood cells II

0558 RECOMBINANT ERYTHROPOIETIN AS TREATMENT FOR THE HYPOREGENERATIVE ANEMIA OF HEMOLYTIC DISEASE OF THE NEWBORN

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Background. Intrauterine transfusions (IUTs) and red cell transfusions (RCTs) and exchange transfusions (EXTs) are usually administered, in utero or during the first days after delivery, for the treatment of hemolytic disease of the newborn (HDN) due to Rh or ABO incompatibility. These transfusional practices induce a severe suppression of the erythropoiesis evidenced by reticulocytopenia, bone marrow erythroid hypoplasia, and inadequately low levels of serum erythropoietin (EPO). Spontaneous reactivation of erythropoiesis only occurs after 2-4 months of age. Consequently, a late hypopregenerative anemia gradually develops between 2nd and 6th weeks of life, and affected infants frequently
require RCTs. A few authors reported that recombinant EPO (rHuEPO) is useful for its treatment and prevention in neonates with Rh HDN who received IUTs or intrauterine EXTs. Furthermore, since the neonatal bone marrow is not able to sustain an adequate erythropoietic response throughout several weeks, this hypoprogenative anemia frequently develops in infants with severe ABO or Rh HDN who received no transfusion. No trial involving this population has been published. Aims. To evaluate the efficacy of rHuEPO for the treatment of HDN due to Rh, ABO or other antigens incompatibility, regardless of whether patients received or not IUTs, RCTs, or EXTs. Methods. After the first week of age, infants started treatment with epoetin α (Hemax®), 250 U/kg, subcutaneously, 3 times a week, when their hematocrit (Htc) dropped to levels requiring RCT, with a clear inadequate reticulocyte response. Patients were closely monitored throughout the following days and RCTs were administered, according to the criteria of the treating physician, if a further decrease of Htc occurred or if clinical signs and symptoms of anemia developed. The treatment was discontinued when the Htc reached normal levels. All patients were given iron (6 mg/kg/day) and folic acid (1.2 mg/day). Results. Twenty-six infants were included (Rh HDN:19; ABO HDN:6; Kpa HDN:1); 9 patients (ABO=6, Rh=3) had not been administered any IUTs, EXTs or RCTs, and 8/19 Rh HDN had received IUTs. Mean age at starting the treatment was 27.5±15.8 days (range: 5-65). Htc and reticulocytes count (Rct) showed significant increases after 7 and 14 days of treatment (Table).

Table 1.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Hematocrit(%)</th>
<th>Ret-He count (%)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Initial Day 7</td>
<td>Day 14 Initial</td>
</tr>
<tr>
<td>All Patients</td>
<td>24.6</td>
<td>27.8* 30.0*</td>
</tr>
<tr>
<td>Rb=Rh Patients</td>
<td>27.5</td>
<td>32.7* 35.9*</td>
</tr>
<tr>
<td>ABO Patients</td>
<td>26.5</td>
<td>28.6* 31.8*</td>
</tr>
</tbody>
</table>

*p<0.001; *p<0.01; **p<0.05; NS

No difference was observed between infants with Rh or ABO HDN. Comparison between patients with Rh HDN receiving or not IUTs showed no significant difference for: a) the Htc increase from day 0 to 7 (7.4±5.8% vs. 3.0±2.8%, respectively), or from day 0 to 14 of treatment (6.6±7.9% vs. 4.8±5.5%, respectively); b) the Htc increase from day 0 to 7 (7.2±5.8% vs. 5.2±3.3%, respectively), or from day 0 to 14 of treatment (7.9±7.4% vs. 5.5±4.7%, respectively). Five neonates (19.2%) required one RCT at days 2, 3, 7, 16 and 24 of treatment (ABO=1, Rh with IUT=3, Rh with no IUT=1). The rHuEPO was administered during 14 to 94 days (mean 37.8±17.7 days). No adverse effect was observed. Conclusion. The rHuEPO seems to be a safe and useful therapy for the late hypoprogenative anemia of HDN due to Rh or other incompatibilities, regardless the administration or not of IUTs or RCTs.

0560

DORSAL SURAL NERVE CONDUCTION STUDY IN VITAMIN B12 DEFICIENCY WITH MEegaloblastic ANEMIA

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Backgrounds. Peripheral neuropathy is frequently observed in B12 deficiency. In spite of this, knowledge about peripheral neuropathy in B12 deficiency is little because the severity of clinical involvement of the central and nervous system clearly outweighs signs and symptoms due to peripheral nervous system involvement. Aims. We primarily investigated peripheral neuropathy with dorsal sural nerve conduction study, which is a new method for detection of early peripheral neuropathy, in B12 deficiency with megaloblastic anemia. Also, as posterior column involvement is the most frequent reported and complicated neuropathy in B12 deficiency, tibial sensory evoked potentials (SEPs) were studied in all patients. Methods. Twenty-eight B12 deficiency patients (15 male, 13 female, mean age 65.8 years) with megaloblastic anemia and 18 age-and-sex matched controls were included. Dorsal sural nerve conduction studies, conventional motor/sensory nerve conduction studies and tibial SEP were performed. Results. Although dorsal sural sensory nerve action potentials (SNAPs) were not recorded in 15 (54%) of 28 patients, only 9 (32%) of them had polynuropathy with conventional conduction studies. Furthermore, patients with dorsal sural SNAP had mean lower amplitude, mean longer latency and slower velocity response when compared to controls. Twenty patients (71%) were diagnosed with myelopathy with the combination of tibal SEP and neurological findings. Two patients whose dorsal sural SNAP were not recorded had normal tibal SEP responses; therefore, these patients were considered to have isolated peripheral neuropathy. Summary/Conclusions. As a result, we conclude that dorsal sural nerve conduction study is a reliable method for detection of early peripheral neuropathy in B12 deficiency. On the other hand, in concordance with previous studies, dorsal tract involvement is more common than neuropathy in B12 deficiency.

0561

ANTI-CARDIOLIPIN, Anti-B2 GLYCOPEPTIDE I, AND Anti-PHOSPHATIDYL-SERIN AUTO-ANTIBODIES AND THE RISK OF SICKLE CELL ANEMIA

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Background. Anti-cardiolipin (ACA), anti-β2 glycoprotein I (anti-β2GPI), and anti-phosphatidylserin antibodies (APS) were associated with thrombophilic disorders and hypercoagulable states, including deep venous thrombosis, recurrent pregnancy loss, and stroke. Aims. We determined the prevalence of these autoimmune antibodies as a risk factor for sickle cell anemia (SCA) among Bahraini patients and control subjects. Patients/Methods. This was a case-control study; study subjects comparing 78 SCA patients (mean age: 15.8±9.8 years) diagnosed with SCA according to hemoglobin profile, and 88 control subjects (mean age: 27.8±15.2 years) with no history oh hemoglobinopathies. ACA, β2GPI, and APS IgM levels were determined with ELISA. An ACA cut-off was set as the mean value ±3 SD of control subjects for each antibody. Results. Patients were matched to controls with respect to gender (p=0.073). ACA IgG (15.4% vs. 2.9%; p=0.01; OR = 6.09; CI = 1.51-28.71), and IgM (11.5% vs. 1.4%; p=0.02; OR = 8.87; CI = 1.09-71.92) were significantly higher among patients than in controls. Anti-β2GPI IgG (24.7% vs. 3.4%; p<0.001; OR = 9.27; CI = 2.61-32.97), but not IgM (8.2% vs. 2.3%; p=0.142; OR = 3.85; CI = 0.75-19.69), were higher among patients than control subjects. In contrast, the prevalence of anti-β2GPI IgG (6.2% vs. 2.7%; p=0.166; OR = 3.22; CI = 0.63-16.53),
and IgM (4.2% vs. 1.6%, p=0.623; OR = 2.69; CI = 0.27-26.56) were comparable between SCA patients than in controls. Summary/Conclu-
sion. ACA IgG and IgM and APS IgG are strongly associated with SCA
sion.
comparable between SCA patients than in controls.
structure, with a particular focus on Neu5Gc residues.
To produce an erythropoietin in a human cell line and characterize the
tests show that individuals have circulating antibodies to Neu5Gc.
glycosylation patterns are thought to affect bioavailability, pharmacoki-
tics and functionality. Of particular interest is the presence of N-gly-
glycosylation patterns are important as, in some bioactive substances (including growth factors),
greatly from that of endogenous human erythropoietin. This may be
Ovary (CHO) cell lines. This leads to glycosylation patterns that differ
a high potency towards reticulocyte pr oduction. It contains the full
nant erythropoietins was also assessed for comparison using the same
quantified following labelling of the released glycans by reverse-phase
sequencing, peptide mapping with reverse-phase, high-performance liq-
tion of a human cell line with DNA containing the appropriate target-
ing and gene-activating sequences. A variety of techniques have been
ied to characterize the resultant erythropoietin including: amino acid
References: Aims: the bibliographic references correspond to the
Aims. Ar

Figure 1. Technique used to produce epoetin delta.
EVALUATION OF MYOCARDIAL IRON DEPOSITION ASSESSED WITH M.R.I. IN YOUNG THALASSAEMIC PATIENTS RECEIVING ONE YEAR OF DEFERASIROX VERSUS DEFEROXAMINE

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Deferasirox (Exjade®) is a new once-daily, oral iron chelator, recently approved by FDA, while is awaiting EU regulatory approval. A multicenter clinical trial, recently published, indicated that daily administration of deferasirox at a dose of 20 mg/kg maintains iron concentration (LiC), whereas doses of 30 mg/kg achieve significant LiC reduction. Aim of this study was to evaluate the effectiveness of deferasirox in removing iron from the heart in comparison to deferoxamine, with the use of Magnetic Resonance Imaging (M.R.I.). In our center 11 young patients with β-thalassemia major, aged 10 to 16.5 years with a mean age of 14.2±2.5 years, participated in a large multicenter, Phase III, comparative trial of deferasirox versus deferoxamine. Seven patients were randomized in the deferasirox group and 4 in the deferoxamine group. Doses were assigned according to baseline LiC assessed with percutaneous liver biopsy. In line with previous clinical management at our center, these patients were studied with myocardial M.R.I, as part of their routine monitoring, at the begging of the trial and one year after. MR images of heart were acquired during systolic phase, using electrocardiogram-triggered, flash 2D sequences, with 5 mm thickness and FOV 360-240mm. Region of interest (ROI) measurements were performed in the air and in the left ventricular myocardium. The natural logarithm of the signal intensity of the studied tissue to air ratio [ln (mean signal intensity of tissue / SD of air)], was calculated, with estimating normal values above 3.2. All patients completed one year of the study with no major adverse event. None of them was presented with any symptoms of cardiopathy, and heart echo, routinely performed according to the protocol design, was normal in every patient. All M.R.I’s values were within normal range, something which can be attributed to the young of age. Mean heart M.R.I values at the begging of the study were 4.29 for the deferasirox and 4.3 for the deferoxamine group.

### Table 1

<table>
<thead>
<tr>
<th>Agent</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>30 mg/kg</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>MRI start</td>
<td>4.21</td>
<td>4.37</td>
<td>3.98</td>
<td>4.29</td>
</tr>
<tr>
<td>MRI end</td>
<td>3.60</td>
<td>4.25</td>
<td>4.64</td>
<td>4.21</td>
</tr>
<tr>
<td>p-value</td>
<td>0.17</td>
<td>0.32</td>
<td>0.30</td>
<td></td>
</tr>
</tbody>
</table>

One year after, heart M.R.I values were 4.21 and 4.41 respectively with no statistically significant difference. Of particular interest is the fact that one patient receiving deferasirox at a low dose of 10 mg/kg showed a significant reduction in M.R.I values (4.21 versus 3.60), whereas patient receiving high dose of 30 mg/kg managed to reduce myocardial iron deposition as indicated by the significant increase of M.R.I values (3.96 versus 4.64). Results are shown on the following Table. In conclusion, deferasirox at a daily dose of 20 mg/kg seems to be equivalent to deferoxamine doses of 40-50 mg/kg in maintaining myocardial iron concentrations. Similarly to liver, effect of deferasirox in removing myocardial iron is dose-dependent Low dose of deferasirox (10 mg/kg) seems to be ineffective, whereas one patient receiving high dose showed an encouraging improvement in myocardial M.R.I values. Randomized, controlled studies are needed for safer conclusions.

DIVERSE MOLECULAR DEFECTS ASSOCIATED WITH IDIOPATHIC ERYTHROCYTOSIS REFLECT THE HETEROGENEITY OF THIS DISORDER

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Background: Idiopathic erythrocytoses are a heterogeneous group of disorders characterised by an absolute increase in the red cell mass and associated with variable erythropoietin (Epo) levels. The diagnosis of idiopathic erythrocytosis (IE) is one of exclusion in patients who do not...
fulfil the criteria for the myeloproliferative disorder of polycythaemia vera (PV) and have no identified secondary causes such as a high affinity haemoglobin or Epo producing tumour. The recent discovery of the universal Janus Kinase (JAK2) mutation, V617F, associated primarily with MPD, now makes it possible to identify a clonal stem cell defect in those individuals who previously would have not fulfilled the criteria for PV and thus were included in the IE group. In the majority of IE cases the molecular defect is undefined. Aims. To identify the underlying genetic defects in IE individuals and establish if any of this group of patients would be positive for the PV associated V617F JAK2 mutation. Methods. DNA samples were prepared from more than 120 British and Irish erythrocytosis patients and PCR-direct sequencing of the following genes was performed: the cytoplasmic region of the epo receptor (EpoR), all three exons of the von Hippel Lindau (VHL) protein and the catalytic domain of the prolyl hydroxylase PHD2. Results. Sequencing the EpoR identified a teenage boy with a truncation mutation, G6002A, which removed the terminal 70 amino acids from the receptor. This same mutation was first described in a Finnish skier but microsatellite analysis indicated that both mutations had arisen independently. Screening for the Chuvash VHL mutation, Arg200Gln, in the IE group detected 8 families from the Indian sub-continent who had members homozygous for this mutation. In addition, two Caucasian individuals both with erythrocytosis, D1 and E1, were heterozygous for the same mutation. Although E1 also possessed the G144R VHL mutation, D1 did not exhibit a second defect in the VHL gene but expressed the wild type allele. Most recently, a novel mutation, C950G, in PHD2 has been identified to cause erythrocytosis in 3 members of one family due to an aberration in the Epo negative feedback pathway. Finally, 64 IE individuals were screened by ARMS-PCR to indicate the prevalence of the V617F JAK2 mutation and only one individual was found to possess this mutation. Summary. Although mutations in the oxygen sensing pathway represent the major identified cause of IE so far, they account for only a minor proportion of the total number of patients with IE. Thus the molecular basis of a significant cohort of patients remains elusive but these individuals are a valuable resource that may provide insights into the mechanisms regulating red cell haemostasis and oxygen sensing.

**0568 CHARACTERISATION OF BONE MARROW POSITIVE MITOGEN-STIMULATED DIRECT ANTIGLOBULIN TEST IN PATIENTS WITH REFRACTORY ANAEMIA**

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**Background.** Autoimmune phenomena, particularly directed against RBC, are described in Myelodisplastic syndromes (MDS). We already reported positive BM cultures in patients with refractory anemia (RA) and RA with ringed sideroblasts (RARS) by a new method named mitogen-stimulated-direct antiglobulin test (MS-DAT). Aims. We characterised the target BM cell of the MS-DAT positivity in MDS patients.

**Methods.** MS-DAT was performed by stimulating BM cells with PMA and PHA and antibodies were detected in supernatants by competitive solid phase ELISA. BM cells were separated by magnetic beads in CD45+- (myeloid cells) and CD45- (erythroblasts) and supernatants of positive and negative cultures tested on both BM populations. Results. Eleven out of 23 patients showed positive MS-DAT in BM (cut off value 150 ng/ml ±3SD) and positive patients had increased erythroblast counts and signs of hemolysis (i.e. higher reticulocytes, indirect bilirubin, LDH, and lower haptoglobin) compared with MS-DAT negative ones. Results. The supernatants of BM MS-DAT positive patients showed higher positivity on CD45- autologous BM cells, compared with CD45+ and RBC (Figure 1A). The reactivity was directed both against autologous and control allogenic erythroblasts (Figure 1B). MS-DAT negative BM supernatants had negligible reactivity with CD45- cells both from BM MS-DAT positive and negative patients. Conclusion. Our results show an autoimmune reactivity against erythroblasts in RA and RARS patients with peripheral signs of hemolysis.

**0569 SERUM PRO-HEPCIDIN AND IRON STATUS IN THALASSAEMIA**

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**Background.** Hepcidin is an antimicrobic-like hormone peptide synthesized in the liver. It seems to be a key regulator of iron homeostasis inhibiting intestinal iron absorption, recycling iron in the macrophages and mobilizing iron from hepatic stores. Hepcidin expression is induced by iron overload and inflammation and is suppressed by anaemia and hypoxia. Prohepcidin is a small plasma peptide believed to be a hepcidin precursor. Thalassaemia syndromes are a heterogeneous group of inherited anaemias resulting from reduced or absent synthesis of α- or β-globin chains of haemoglobin, where hepcidin is regulated by opposing factors such as ineffective erythropoiesis, anaemia and iron overload. In these conditions iron overload is mainly due to blood transfusions as well as to increased iron absorption. Aim. To investigate serum pro-hepcidin in a cohort of Thalassaemia Major (TM) and Thalassaemia Intermedia (TI) patients and to evaluate a possible relationship with iron status. Patients and Methods. Thirty-three TM regularly transfused patients, twelve TI patients and twelve normal subjects were studied. TI patients had no or very few transfusions during their life, the last one being at least 5 years ago. Blood from TM was taken at least 48 hours after chelation therapy and just before blood transfusion. Iron status was evaluated by ferritin, percentage of transferrin saturation and non-transferrin bound iron (NTBI). Serum ferritin was determined by standard procedures; NTBI was assayed in serum by HPLC after nitrilotriacetic acid (NTA) chelation. Serum pro-hepcidin was measured by ELISA competitive binding assay (DRG,Germany). Results. Positive correlations were found between pro-hepcidin and ferritin (r=0.423, p<0.01), and between pro-hepcidin/ferritin ratio and NTBI (r=0.356, p=0.01) in TM patients. We report the results in Table 1.

**Table 1.**

<table>
<thead>
<tr>
<th>SF (ng/mL)</th>
<th>NTBI (%maxM)</th>
<th>Pre-hepcidin (ng/mL)</th>
<th>Hb (g/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TM Pre-transfusion (n=43)</td>
<td>875±650^a</td>
<td>0.86±1.23^a</td>
<td>453±366^a</td>
</tr>
<tr>
<td>TI (n=12)</td>
<td>1183±777^a</td>
<td>0.46±1.59^a</td>
<td>546±395^a</td>
</tr>
<tr>
<td>Normal Subjects</td>
<td>170±80</td>
<td>±0.7±0.53</td>
<td>15±44</td>
</tr>
</tbody>
</table>

*p<0.0002 vs normal subjects; ^p<0.0008 vs normal subjects; *p<0.0001 vs TM normal subjects

Conclusions. In thalassaemia pro-hepcidin levels were increased for the degree of iron load and for the possible effect of concomitant minor infections. In thalassaemia syndromes where iron overload and anaemia have opposing effect, the increased erythropoietic stress and iron influx influence hepcidin production. Understanding the mechanisms of iron homeostasis in patients with thalassaemia is of great significance in understanding the pathogenesis of iron load and planning novel treatments.
Conclusions. The results of this ongoing study have shown that the frequency of PH in our cohort of Hbs/β-thal patients is similar with that observed in patients with sickle cell disease. The correlation between PH with reticulocyte counts and ferritin suggests that the degree of hemolysis and iron overload may be implicated in the pathogenesis of PH in Hbs/β-thal. There was no correlation between serum BNP or LDH and the presence of PH; however, this may reflect the number of patients available in the present study.

Table 1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patient with PH (n=15)</th>
<th>Patients without PH (n=39)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (median; range)</td>
<td>41(19.6-60)</td>
<td>36(13-69)</td>
<td></td>
</tr>
<tr>
<td>Gender (n)</td>
<td>7M/6F</td>
<td>12M/27F</td>
<td></td>
</tr>
<tr>
<td>On hydroxyurea (n)</td>
<td>11(46.1%)</td>
<td>15(46.1%)</td>
<td>0.92</td>
</tr>
<tr>
<td>Hb (g/dL; mean±SD)</td>
<td>9.1±1.4</td>
<td>8.8±1.5</td>
<td>0.31</td>
</tr>
<tr>
<td>Retics (x1000/mm³; mean±SD)</td>
<td>230±66</td>
<td>175±65</td>
<td>0.01</td>
</tr>
<tr>
<td>LDH (IU/L; mean±SD)</td>
<td>772±359.7</td>
<td>762±73±340.7</td>
<td>0.46</td>
</tr>
<tr>
<td>Bilirubin (mg/dL; mean±SD)</td>
<td>2.3±1.7</td>
<td>2.4±1.2</td>
<td>0.45</td>
</tr>
<tr>
<td>Creatinine (mg/dL; mean±SD)</td>
<td>0.7±0.1</td>
<td>0.8±0.3</td>
<td>0.10</td>
</tr>
<tr>
<td>Ferritin (µg/L; mean±SD)</td>
<td>1192±6114.2</td>
<td>449±694.8</td>
<td>0.02</td>
</tr>
<tr>
<td>BNP (pg/mL; mean±SD)</td>
<td>202±226.2</td>
<td>310±656.6</td>
<td>0.18</td>
</tr>
<tr>
<td>Hf(%)</td>
<td>16.9±3.3</td>
<td>13.1±2.8</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Background. Echocardiographic studies have reported that approximately 30% of screened adult patients with sickle cell anemia have pulmonary hypertension (PH) defined as systolic pulmonary artery pressures of above or equal to 55 mm Hg or regurgitant jet velocity (TRV) of above or equal to 2.5 m/sec. PH is increasingly observed in hemolytic anemias, including sickle cell disease and thalassemia in particular thalassemia intermedia. Brain natriuretic peptide (BNP) is released from the ventricles during pressure strain and its levels would correlate with severity of PH. Aims. The aim of this study was to evaluate the prevalence of PH in correlation with hemolytic findings and BNP levels in a cohort of patients with double heterozygous sickle cell trait and β-thalassemia (Hbs/β-thal). Methods. We studied 52 patients (19 males and 33 females) with Hbs/β-thal (thal 0: 35 pts and thal ±: 17 pts) who were followed up regularly in the Thalassemia Center of Laikon Hospital. Their median age was 55 years (range: 21-62 years). All pts were evaluated for the presence of PH using Doppler echocardiography and then applying the modified Bernoulli equation (Pulmonary artery systolic pressure=4V² + right atrial pressure). Exclusion criteria of this study include: 1) evidence of left ventricular failure; 2) vaso-occlusive crisis during the last 15 days; 3) atrial fibrillation or ventricular tachycardia; 4) mitral value regurgitation (MVR) >2/4+ or mitral value stenosis; and 5) severe pericardial perfusion. In all patients we measured Hb, leucocyte and platelet counts, reticulocyte counts, serum lactate dehydrogenase (LDH), bilirubin, ferritin, creatinine, Hb F and BNP levels. Twenty-four (46%) patients were on hydroxyurea administration for a median time of 4+5.5 years. Results. Thirteen (25%) patients had PH, according to established criteria. All patients had mild symptoms, such as fatigue or dyspnea on slight exertion. The administration of hydroxyurea did not establish criteria. All patients had mild symptoms, such as fatigue or dyspnea on slight exertion. The administration of hydroxyurea did not establish criteria.
quality of life and satisfaction of patients who are currently undergoing iron chelation therapy. Method. The Italian Thalassemia Cost and Outcome Assessment (ITHACA) is a naturalistic, multicenter, retrospective study involving patients with β-thalassemia major of any age and who are on iron chelation therapy for at least 5 years, sequentially enrolled at 8 Italian Thalassemia Care Centers. Information on socio-demographic, clinical, resource utilization, quality of life and treatment satisfaction was collected as a battery of questionnaires. The aims of this study were to clarify the aberrant hemocytometry and hemoglobin variant results in patients with thalassemia, to identify any laboratory tests that are applied in routine practice and to study these hemoglobin disorders. In routine practice, the laboratory tests most commonly applied are the determination of hemoglobin. Hemocytometry and hemoglobin variant analysis are the laboratory tests to study these hemoglobin disorders. In routine practice related to thalassemia (mean number of complications per medical costs were evaluated. Overall, the average cost was €1162.3 €/patient/month, the major cost driver was chelation therapy including drugs and administration costs, and it represented 53.1% of total costs, followed by transfusions (35.6%), surgical interventions (5.6%), laboratory and instrumental tests carried out in outpatient or day hospital setting and medical visits (3.3%), concomitant medications (1.7%). Direct non-medical costs, such as transportation, represent 2.9% of total cost. By age category, the daily cost of infusion with Desferal was estimated to approximately 22 € excluding pump and consumables. Conclusions. This is the first study to assess the cost of care of patients with β-thalassemia major in Italy. Our findings show that transfusion and iron chelation procedures together account for the great part of short term health care cost. These results can be considered informative since consumables, nursing, home care and long-term complication costs are not included. Studies are needed to estimate indirect and long-term cost of thalassemia major in order to obtain a more accurate and broader picture.

0573 A FAMILY WITH A MILD PHENOTYPE DESPITE MULTIPLE MUTATIONS IN THE α- AND β-GLOBIN GENE CLUSTER
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Background. Hemoglobinopathies refer to a diverse group of disorders caused by an abnormal structure of the hemoglobin molecule. Thalassemias are hereditary disorders characterized by defective production of hemoglobin. Hemocytometry and hemoglobin variant analysis are therefore used to study these hemoglobin disorders. Diagnostic, molecular analysis is not standardly applied. We describe a family with a generally mild anemic phenotype despite multiple mutations in the α- and β-globin gene cluster. Aims. The aim of this study was to clarify the aberrant hemocytometry and hemoglobin variant results of the proposita and her family by means of molecular analysis. Methods. First, DNA was extracted using the QIAamp DNA Blood 1000 (Abbott). Hemoglobin variant analysis was performed by capillary electrophoresis (Biorad). DNA sequence analysis was performed using the ABI 310 genetic Analyzer (Applied Biosystems). Results. The proposita (female, age 20) was referred to our laboratory upon suspicion of thalassemia (Hb 9 mmol/L, MCV 72 fl, MCH 1.62 fmol, erythrocytes 5.56x1012/L). She complained of fatigue but had no other clinical symptoms. Quantification of hemoglobin variant showed 113.8% HbA1, 4.4% HbA2, 58.9% HbC, and 25.5% HbF relative to total hemoglobin. On one allele of the patient we detected an HbC mutation (codon 6 GAG to AAG in ciss with the non-functional γα and 1G promoter sequence) in the β-globin gene (271C>T). In the other allele a β-thalassemia promoter mutation that is known to cause β-thalassemia and two novel mutations in the promoter of the γα gene (271C>T) and γγ gene (403_592delCTTAAA) were detected. Further analysis of the patient’s α-globin gene cluster revealed also the presence of a heterozygous β-thalassemia (5.7 deletion). DNA analysis of four family members revealed, in addition, several other sequence deviations in the γ-globin genes. Three common mutations were detected in the γα gene promoter: 222_226del(A)AGCA(A), 309A>G, and 369G>C, whereas one novel mutation was detected in the γα gene promoter: 497T>A. The first of these mutations is known to lower γ-globin expression, the second is associated with increased HbF levels in normal healthy adults, the third is not associat with increased HbF levels in normal adults, and the functionality of the latter is unknown at present. Conclusions. In the here presented family a total of ten different mutations were found in the globin genes: one mutation in the β-globin gene and seven in the α-globin genes. In spite of this, the proposita and all family members displayed a mild clinical phenotype. It is possible that, ultimately, this beneficial mild phenotype results from the interplay between the various identified genetic variants which function as phenotypic modifiers. This case shows that molecular analysis subsequent to biochemical analysis can be beneficial, but that the need for such analysis should always be considered in relation to the clinical practice.

0574 GENOMIC INSTABILITY IN SICKLE CELL DISEASE PATIENTS USING HYDROXYREA ASSESSED BY ALKALINE SINGLE-CELL GEL ELECTROPHORESIS ASSAY
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Hydroxyurea (HU) is considered and antineoplastic drug, which also plays an important role in treatment of sickle cell disease patients (SCD). Short-term HU toxicities primarily include transient myelosuppression, but long-term HU risks have not been defined. The mutagenic and carcinogenic potential of HU is not established, although HU has been associated with an increased risk of leukemia in some patients with myeloproliferative disorders. In the present study we analyzed the presence of DNA damage in patients with SCD treated with HU, in peripheral blood lymphocytes, using alkaline single-cell gel electrophoresis assay. We analyzed 36 patients with sickle cell disease (16 males and 20 females), aged 2-59 years (mean 25.75±14.45), received oral HU median dose of 26.5 mg/kg/day, for a period of 0.6-11.3 years (mean 5.01). The control group was composed of 23 healthy individuals (10 males and 13 females), aged 4-52 years (mean 26.61±7.20). The results revealed that damage index in SCD patients was significantly higher than in controls (p=0.0014). This study indicates a potential genotoxicity of the HU, although further works are necessary to evaluate its mutagenicity.

0575 DISTINCT PHENOTYPIC EXPRESSION IN PATIENTS WITH THE HYPERUNSTABLE ALPHACHAIN VARIANTS HB TAYBEE (ALPHA1CD38/39DEL/ACC; THR-0) AND HB HERAKLION (ALPHA1CD36/37DEL/CC; PRO-0)
P. Papassotiriou,1 E. Kanavakis,2 D. Liapi,1 A. Tsilimigaki,1 A. Stamoulakatou,1 M. Mavrokosta,1 J. Traeger-Synodinos1 1Aghia Sophia Children’s Hospital, ATHENS, Greece; 2Medical Genetics, Athens University, ATHENS, Greece; 3‘Ventzillon’ Hospital, HERAKLION, Greece

α-thalassemia mutations are of the commonest in humans. Amongst more than 80 α-thalassemia mutations, more common are deletions, partially or completely removing the α-globin gene cluster (16p13.3, G2- ψα1-α2-α1-ψ1-α2-α1-ψ1-α2-α1-ψ1-α2-α1-ψ1-α2-ψ1-α2-α1-ψ1-α2-α1-ψ1-α2-α1). Point mutations within either α-globin gene (non-deletion mutations) are less common. Non-deletion mutations usually reduce α-globin synthesis by impeding RNA processing or translation, but some cause post-translational hyper-instability of the abnormal polypeptide, mimicking α-thalassemia through an overall reduction of α-globin synthesis. Interaction of non-deletion α-thalassemia determinants usually causes Hb H disease, a chronic moderate anemia in which excess β-globin chains form Hb H (β4-). We observed 6 Greek cases with an atypical β-like thalassemia phenotype: chronic moderate anemia without abnormal hemoglobin fractions. DNA analysis characterized the α-thalassemia mutations in-trans and/or in-frame deletions in the α-globin gene: 4 cases (2 unrelated children and 2 adult siblings) had codon63/57, delCCC (Hb Heraklion), and a further 2 unrelated adults had codon58/59, delACC (Hb Taybee), which represents the first observation of Hb Taybee in Greece. Three Hb Heraklion cases with the non-deletion IVS-1 donor splice-site mutation (εHPhoX) in trans had Hb levels 70-95 g/L; the fourth Hb Heraklion case and both Hb Taybee cases had α-thalassemia 3.7kb deletion in trans, maintaining Hb levels around 105 g/L. All cases had slight to moderate hemolysis (bilirubin 3 to 7-fold normal) and increased erythroid marrow activity indicating dyserythropoiesis (serum erythropoietin and soluble transferrin receptor levels 5 to 8-fold normal). Furthermore parameters indicating the status of oxidant-antioxidant balance showed moderate impairment of the glutathione system and an increased rate of lipid peroxidation, reflected by increased levels of malondialdehyde. Absence of detectable abnormal hemoglobin fractions (including Hb H) in such cas-
es confounds diagnosis based on hematology alone, and definitive diagnosis is only achieved through DNA analysis. To date Hb Heraklion has been observed in a single Greek case and Hb Taybeh in sporadic Israeli-Arab cases. There is minimal experience for the management of such atypical cases, and our previous experience indicates that it is probably insufficient to monitor clinical status in patients with hemoglobinopathies based on hemoglobin levels alone.1

Reference

0576 EPOETIN DELTA, ERYTHROPOIETIN PRODUCED BY A HUMAN CELL LINE, IS EFFECTIVE IN THE TREATMENT OF RENAL ANAEMIA
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Background. Several recombinant erythropoietins are currently available for the treatment of anaemia associated with chronic renal failure and cancer. All of these agents are produced in Chinese Hamster Ovary cell lines and as a result have glycosylation patterns that differ from endogenous erythropoietin. Epoetin delta (Dynepo®, Shire) is an erythropoietin produced in a human cell line through gene activation. Aims. To assess the efficacy and safety of different doses of epoetin delta in patients with anaemia and chronic renal failure requiring haemodialysis. Methods. In this multicentre, double-blind study, haemodialysis patients with anaemia (haemoglobin < 10.0 g/dL) who had not previously received an epoetin were randomized to receive epoetin delta (15, 50, 150 and 300 IU/kg) or epoetin alfa (50 IU/kg) three times a week. In the initial correction phase, patients received the allotted dose until ‘correction success’ was reached (two consecutive weekly haemoglobin measures ≥ 11.5 g/dL or one measurement ≥ 15 g/dL). Patients achieving correction success then entered a maintenance phase, during which the dose was titrated to maintain haemoglobin levels at ≥ 10.5 g/dL. The maximum duration of treatment was 12 weeks. Maintenance success was defined as haemoglobin ≥ 10.5 g/dL at week 12. Total success was defined as achievement of both correction success and maintenance success. Data from the groups assigned the two highest doses of epoetin delta were pooled and compared with results from the lowest dose group. Results. In total 78 patients were randomized and 75 received treatment (epoetin delta 15, 50, 150 and 300 IU/kg; 21, 14, 13 and 13 patients, respectively; epoetin alfa 50 IU/kg; 14 patients). Baseline haemoglobin levels were similar in the pooled epoetin delta and epoetin alfa groups (8.66±0.94 and 8.57±0.82 mg/dL). The proportion of patients achieving total success was higher in the pooled highest dose epoetin delta group (150 and 300 IU/kg) compared with the lowest dose (15 IU/kg) group (55.6% vs. 4.5%; p=0.0002). Analysis of dose trend across the epoetin delta groups showed a significant trend for an increase in total success and correction success with increasing doses (15, 50, 150, 300 IU/kg: total success, 4.5, 21, 4, 50, 61.5%, respectively, p=0.0001 for trend; correction success, 9.1, 21.4, 57.1, 61.5%, respectively, p=0.0002 for trend). There were no significant differences in success rates between epoetin delta 50 IU/kg and epoetin alfa 50 IU/kg. The incidence of treatment-emergent adverse events was similar in the epoetin delta and epoetin alfa groups. Adverse events thought to be possibly related to epoetin delta occurred in 11.5% of patients and most were mild or moderate in severity. There was no evidence of dose-related adverse events. Conclusions. Epoetin delta is effective in increasing haemoglobin levels in patients with haemoglobin < 11g/dL as a result of chronic renal failure, and shows at least similar efficacy to epoetin alfa at an equivalent dose. Safety profiles were similar for the two agents. No patient receiving epoetin delta developed anti-erythropoietin antibodies.

Cytogenetics and Molecular Cytogenetics

0577 ONCOGENIC DEREGULATION OF HOXA GENES BY CHROMOSOMAL REARRANGEMENTS IN T-CELL LYMPHOPROLASTIC LEUKEMIAS (T-ALL)
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T-cell acute lymphoblastic leukaemias (T-ALL) are highly malignant tumours which derive from partially differentiated T-cell progenitors. We recently reported the identification of a new recurrent chromosomal rearrangement in human T-ALL, targeting the major homeobox gene cluster HOXA and the T-cell receptor genes locus TCRB (Soulier et al., Blood 2005). This rearrangement was found in four patients out of a series of 92 T-ALL and corresponded to inv(7)(p15q34) and t(7,7) (p15q55), in 5 and 2 cases, respectively. The 4 HOXA breakpoints were analysed at the molecular level by Southern blot in the 4 cases, and cloning of the two derivative breakpoints in two cases. The breakpoints clustered within a 2.6 kb region in the HOXA locus. In order to analyse the molecular consequences of this rearrangement, the expression of the 11 HOXA genes was analysed on micro-array data and by specific RT-PCR. We found that the whole HOXA gene cluster expression was deregulated in the rearranged cases, on both side of the breakpoint cluster region, compared to other T-ALL. Mechanisms of this deregulation remain elusive. Two additional groups of T-ALL demonstrated a global HOXA cluster deregulation, namely the CALM-AF10 and the MLL-rearranged T-ALL cases. These results strongly suggested that the deregulation of HOXA genes is oncogenic in T-ALL. Global gene expression analysis and unsupervised hierarchical classification in the 92 cases T-ALL series demonstrated that the TCRB-HOXA associated cases clustered in an homogeneous subgroup, which shared common expression profile with the TLX1/HOX11 and TLX3/HOX2 associated subgroups. This suggested use of common biological oncogenic pathways in these homeobox genes associated T-ALL. Like other T-ALL, these cases frequently demonstrated NOTCH1 gene activation mutations and CDKN2A/p16/ARF deletions, consistent with multi-events oncogenesis. We then analysed expression of two alternative transcripts, HOXAb and HOXAb10b, considering the clusterization of the breakpoints between the HOXAb9 and the HOXAb10 genes in the TCRB-HOXA translocated cases. Interestingly a massive expression of the HOXAb10b transcript was demonstrated, whereas no significant expression was detected in the CALM-AF10 and MLL-associated T-ALL cases, or in other T-ALL cases. The HOXAb10b transcript has been detected during early embryogenesis in mice and in leukemic cell lines. It encodes a short HOXAb10b protein which retains the homeobox domain of the regular HOXAb10 protein (HOXAb10A) but lacks the N-terminal regulation domain. We found no expression of HOXAb10b during T-cell differentiation by analysing normal human thymus samples, showing that its expression in T-cell leukemic cells was ectopic. Considering the specific expression of the HOXAb10b transcript in the TCRB-HOXA cases, we are currently analysing the phenotypic consequences of the HOXAb10b homeobox gene overexpression in mouse models using retroviral transduction of bone marrow progenitors.

0578 CORRELATION BETWEEN CHROMOSOMAL ABNORMALITIES AND IMMUNOHISTOCHEMICAL PROFILE IN DIFFUSE LARGE B CELL LYMPHOMAS REVEALS DISTINCT LYMPHOMAGENESIS PATHWAYS WITH CLINICOPATHOLOGIC SIGNIFICANCE AND PROGNOSTIC VALUE
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Background. DLBCL constitutes a heterogeneous group. The genetic and molecular mechanisms underlying their diverse clinical presentations and outcomes have been partially clarified by the recent application of DNA microarrays and cDNA expression technologies. Comparative genomic studies of DLBCL also revealed a broad spectrum of clonal genetic abnormalities and complex karyotypes, including, chromosomal translocations, deletions, duplications and other complex alterations. However the potential clinicopathological relevance of these alterations is still poorly defined. Patients and Methods. 101 previously untreated patients
diagnosed with de novo DLBCL at our hospital between 1987 and 2003 were selected (median age = 59 years, 50 males, IPI 0-1: 32%, 2-5: 46%, 4.5: 22%). The inclusion criteria were the availability of appropriate paraffin-embedded tissues and a karyotypic analysis using R-bandit method. Hierarchical clustering analysis based on immunostaining with a large panel of antibodies (including cell-cycle control, apoptosis, immune response and B-cell differentiation markers) was performed and correlated with current cytogenetic abnormalities and outcome. The germinal center B-cell-like (GC) and the non-GCB phenotypes were defined using CD10, BCL6 and MUM1 immunostaining. Results. Among the 101 studied patients, 10 karyotypes were considered as normal. The most frequent numerical genetic abnormalities were monosomy 15 (19%), trisomy 15 (7%), 7 (5%) 11 (17%) (26%) 18 (17%) and Xp (17%). The most frequent structural abnormalities involved 1p (31%), 1q (38%), 2p (21%), 3p (16%), 3q (45%), 4p (21%), 5q (16%), 6q (38%), 7q (19%), 8q (15%), 9p (16%) 11q (15%), 14q23 (49%), and 18q (35%). The t(4;14), t(3;4) and t(8;14) were observed in 21%, 20% and 2% of cases respectively. The GCB phenotype was observed in 60% of cases and is significantly related to t(14;18) (36%), trisomy 12 (56%), and 12p13 (4%) and 2p (3%) rearrangements. The non-GCB phenotype was observed in 54% of cases and correlated with 3p (23%) and 3q (57%) rearrangements. DLBCL with t(4;14) and t(14;18) are preferentially CD10+ (72%), BCL2+ (68%) and MUM1 negative (56%). By contrast, DLBCL with t(3;4) were more often p53+ (41%), MUM1+ (94%), usually expressed the anti-apoptotic protein-3 molecule (70%) but were BCL2 negative (88%). Using an unsupervised hierarchical clustering approach based on the expression of a large panel of antibodies, 82% of cases could be properly reclassified only by considering the presence of a t(4;14) or of a t(3;4), indicating clearly 2 distinct cells of origin. Finally, p53 protein expression correlated with 1p and 3q27 rearrangements and 3q27 abnormalities with a significant unfavorable prognosis impact were the 1p7, 3p and 9p13 rearrangements. A scoring system, including all unfavourable genetic abnormalities was strongly predictive of the outcome, independently of the GCB/non-GCB phenotype and was confirmed in an independent series of 87 DLBCL. In addition to these clonal genetic abnormalities, BCL2, CD5 and p53 expression were associated to a poor clinical outcome. Conclusion. This study demonstrates correlation between chromosomal abnormalities and immunohistochemical profile in DLBCL, and reveals distinct lymphomagenesis pathways with clinicopathologic significance and prognostic value. These results contribute to a molecular database that could allow the identification of new relevant genes involved in lymphomagenesis.

0579

PDGFRB FUSES TO TPM3 IN THE T(1;5)(Q23;Q33) OF CHROMOSOMAL TRANSLOCATIONS COLLECTED IN THE T(5;16)(Q55 ;P13) OF CHRONIC MALIGNANT LEUKAEMIA


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Background. Ph-negative chronic myeloid leukemia and chronic myelomonocytic leukemia (CMML) with bone marrow and/or peripheral blood eosinophilia are associated with PDGFRB or FIP1L1-PDGFRB translocations. The 3′ region of PDGFRB encoding the kinase domain fuses with the 5′ region of a partner gene encoding an oligomerization domain, which determines the constitutive activation of PDGFRB tyrosine kinase. In order to identify PDGFRB transcripts in which the kinase domain is fused to a non-coding sequence, we performed a comparison of the sequences of PDGFRB translocations with those reported in the public databases. We identified several putative PDGFRB fusion partners. Methods. Patient 1, a 21-year-old man with chronic eosinophilic leukemia showed a 46,XX (t(15;16)(q22;q23)) karyotype in 28/29 metaphases. The patient underwent α-IFN and oncorbicide treatment for ten years and switched to imatinib after identification of a PDGFRB rearrangement. Patient 2, a 36-year-old woman with Noonan syndrome and exon 3 of PDGFRB mutated, was admitted because of CMML. Cytogenetic analysis showed the following karyotype: 46,XX(t(15;16)(q22;q23)) [11/46XX]. After assessing PDGFRB involvement in maternally inherited therapy was administered and haematological, cytogenetic, and FISH remission was achieved after six months. FISH with two cosmids for PDGFRB/q53 (9-4 and 4-1) was performed in both patients. DNA clones for the 1q23 band in patient 1, and for 16p13, in patient 2, were applied to narrow breakpoints and to select candidate partners. RT-PCR with gene-specific primers were performed to amplify TPMS/PDGFRB and NDE1/PDGFRB fusions from patients 1 and 2, respectively. Amplions were sequenced for confirmation. Functional assays were performed on the NDE1/PDGFRB fusion by testing transduced Bax/Fas cells for IL-3 independent growth. Sensitivity of the NDE1/PDGFRB fusion protein to imatinib was evaluated. Results. Cosmids for PDGFRB gave a red/green fusion signal on normal 5 and a split signal with cosmid 9-4 and 4-1. Fused transcripts were identified in patient 1 or patient 2. In patient 1, the 1q23 breakpoint fell within clone RP11-205M9. In patient 2, DNA clone CTD-2303E13 NDE1/16p13 and cosmids 27/29 for the 3′MYH11/16p13 were present on normal 16 and on der(5) while cosmids 14/18 for the 3′MYH11/16p13 and clone RP11-8H13 MRPI/16p13 gave one signal on normal 16. In patient 1, RT-PCR for TPMS/PDGFRB amplified a chimeric transcript joining TPM3 exon 7 to PDGFRB exon 11. In the second case, RT-PCR showed fusion between exon 5 of NDE1 and exon 11 of PDGFRB. Ba/F3 cells transduced with NDE1/PDGFRB fusion were transformed to IL-3 independent growth. The NDE1/PDGFRB fusion protein was shown to be sensitive to imatinib. Conclusion. We identified TP3/1q23 and NDE1/16p13 as two new PDGFRB/S53 partners in patients with CEL and CMLM, respectively. Interestingly, the TPM3 gene is already known for its involvement in the t(1;22)(q52;p13)/TPM3-ALK of anaplastic large cell lymphomas and for its ability to fuse with RUNX1 in papillary thyroid carcinomas, while NDE1 haploinsufficiency has been associated with the onset of a MDS-like syndrome in mice. Acknowledgements. DNA clones were kindly provided by Dr. M Rocchi, University of Bari, Italy. Supported by: MIUR, FIRB, AULL, and Fondazione Cassa di Risparmio, Perugia, Italy. B.C. is supported by a grant from FIRC.

0580

A DNA-BASED RQ-PCR SCREENING ASSAY FOR RUNX1 COPY NUMBER CHANGES IN CHILDHOOD B CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. An increased RUNX1 copy number is a common finding in BCP-ALL. It usually identifies additional copies of chromosome 21, which in turn is a typical feature of hyperdiploid cases. In a much smaller proportion of cases, however, it results from rearrangements that specifically multiply the region 21q22 (intra-chromosomal RUNX1 multiplication; ICRM). This distinct genetic marker, also known as AML1 amplification, designates a specific form of BCP-ALL with a pronounced risk of relapse. Noteworthy, less than 100 such cases have been reported worldwide. The aim of the current project was to characterize the quantitative assessment that at present an ICRM can only be detected with fluorescence in situ hybridization (FISH) and a systematic FISH screening is only conducted in very few treatment trials. Aim and Methods. To overcome this diagnostic obstacle, we developed a DNA-based real-time polymerase chain reaction (RQ-PCR) screening assay. It is based on the comparative quantification of three regions of RUNX1 at 21q22, PRSS7 at 21q21.1 (as an intra-chromosomal control) and at 1q21.1 (as an inter-chromosomal control). The assay was set up and validated with DNA from cases with two (normal controls), three (Down syndrome patients) and four (hyperdiploid ALL) chromosomes 21 and put to test on samples from 15 Austrian cases with a previously FISH-verified ICRM. The number of additional RUNX1 copies in these samples was determined to range from 4 to approximately 8. Results. Screening of 221 BCP ALL samples from the German BFM-ALL trial identified altogether 88 cases with a RUNX1 copy number increased by two or more (10%). Two of them had a constitutional trisomy 21, five (2,3%) an ICRM, 65 (29%) were hyperdiploid and 16 (7%) were ETV6/RUNX1-positive and had an extra copy of chromosome 21. Conclusion. The respective PCR results were in good accordance with those suggested by DNA-index, cytogenetic or FISH analyses. They prove that such a DNA-based screening technique can reliably identify and delineate RUNX1 overrepresentations in different genetic BCP ALL sub-forms, such as those with an ICRM, a hyperdiploid or pseudotetraploid karyotype as well as those ETV6/RUNX1-positive ones with secondary changes. Since this approach is extremely well suited for the fast and efficient retro- and prospective analyses of a large number of cases we foresee that it will become the preferred method for the identification of such cases in childhood ALL treatment trials.

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**0581**
META-ANALYSIS OF 966 B-CELL NEOPLASMS WITH 8q24 ABERRATIONS IDENTIFIES DISTINCT CYTOGENETIC ABERRATION PATTERNS OF BURKITT AND NON-BURKITT LYMPHOMAS

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**Background.** Burkitt lymphoma (BL) is cytogenetically characterized by a translocation juxtaposing the MYC locus on band 8q24 next to the IGH locus on 14q32 or one of the light chain loci on 2p12 and 22q11. However, translocations affecting the MYC locus are not exclusive for BL but also occur in other B-cell neoplasias. Aims. Based on published karyotypes derived from conventional cytogenetic analyses, we intended to define the typical cytogenetic signature of Burkitt lymphoma and to delimit this signature from other B-cell lymphomas (B-NHL) with 8q24 translocations. A typical cytogenetic signature could be used as adjunct for clinical diagnosis, and may point towards loci targeted by disease-specific genetic events. Methods. We performed a meta-analysis of karyotypes from 966 B-cell neoplasms from the Mitelman with 8q24 breakpoint in the main clone using software from the Progenetix project. 461 cases were diagnosed as Burkitt lymphoma or leukemia. The remaining cases consisted (in decreasing number) of ALL (NOS), DLBCL, B-NHL (NOS), myeloma, FCL, and other B-NHL entities. Results. 440 BL cases lacked a translocation involving 8q26-q27 or 18q21. Of those typical BL, 256 had chromosomal imbalances (average 1.5 imbalances, median 1 imbalance per case). The 446 non-BL B-NHL with cytogenetic translocations indicating a MYC/IG fusion showed a greater overall chromosomal instability (average 5.7 imbalances, median 3 imbalances). In BL, recurring gains involved 1q21-q32 (21%), chromosome 7 (7%) and chromosome 12 (5%). Losses were rare, with a maximum of 4.5% on 17p. Other regions affected by chromosome aberrations in B-NHL like 3q, 6q, 12q and 18q were rarely imbalanced in BL. No differences were observed for lymphomatous and leukemic variants of BL. Gains on 1q and 12 were nearly exclusive in BL, with co-occurrence in only one case. In contrast, the 21 BL cases with additional 3q26-7 or 18q21 break exhibited typical B-NHL aberrations, such as 8q26 loss (15%) or 18q gain (10%) but no gain on 1q. The non-BL B-NHL cases with cytogenetic translocations indicating a MYC/IG fusion displayed a heterogeneous pattern of imbalances. As in BL, the most common gains involved 1q22-q23 (24.9%), 7 (19.4%) and 12 (10%). However, chromosomes 19p (10.1%), 3 (9.8%), 9q (9.4%) and others (21, 11q, 5, 15) had frequent gains, too. Recurring losses involved 6q21 (11.9%) and 13q (11.9%). In contrast to the BL subset, limitation to single chromosomal imbalances was much less common (87% for 0 or 1 imbalance in BL). The non-BL B-NHL cases with cytogenetic translocations indicating a MYC/IG fusion contain only few, usually single genomic imbalances. The low complexity of BL underscores the etiologic importance of the Ig/MIYC fusion in this disease. The mutually exclusive pattern of imbalances may point to alternative genomic events co-operating with Ig/MYC translocations in BL. BL cases with additional B-NHL abnormalities may be part of a distinct disease group. Non-BL B-NHL with 8q24 translocations display a heterogeneous pattern and larger number of chromosomal imbalances. Our analysis exemplifies the importance of large data collections for determining relevant cytogenetic aberration patterns.

**0582**
MOLECULAR CHARACTERIZATION OF DISTINCT HOT SPOT REGIONS ON CHROMOSOME 7q IN MYELOID LEUKEMIAS

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**Background.** Loss of whole chromosome 7 (-7) or deletion of the long arm (7q-) are recurring chromosome abnormalities in myeloid leukemias. In recent years, several groups initiated the molecular characterization of the deletion and translocation breakpoints. Based on these results a common deleted segment (CDS) of approximately 2 Mb in size was identified in chromosomal band 7q22 flanked by the microsatellite markers D7S1503 and D7S1841. Recently, we mapped the translocation breakpoint of a t(5;7)(p13;q22) within this genomic segment and identified a novel gene (MLL3, mixed lineage leukemia 5) that represents a candidate gene for chromosome 7 associated leukemias. With respect to deletions affecting the distal part of chromosome 7q a to 4 to 5 Mb sized CDS was defined encompassing chromosomal bands 7q35 to q36. However, several other CDS and translocation breakpoints on 7q have been described so far, suggesting the existence of more than one disease-related gene. Aims. To identify and characterize translocation and deletion breakpoints in a large series of myeloid leukemias including acute myeloid leukemia (AML) and chronic myeloid leukemia (CML). Methods. FISH with a physical map of well defined YAC (yeast artificial chromosome) clones representing the long arm of chromosome 7 was performed on a series of 105 myeloid leukemias including acute myeloid leukemia (AML), myelodysplastic syndrome (MDS) and myeloproliferative disorders (MPD). In addition, selected patients were analysed by array CGH and results were confirmed by hybridisation of the corresponding DNA clones. Transcriptional maps were constructed by the use of public databases. Results. While most of the deletions were large and encompassed the previously published CDS, we identified a distinct 2 Mb sized genomic segment in the proximal part of 7q22 that was defined by five patients. This segment contains several candidate genes including the putative tumor-suppressor genes CUTL1 and RASA4. Interestingly, this CDS is located close to multiple miR-sites, which usually indicate common fragile sites in the human genome. In chromosomal bands 7q35-q36 we localized the breakpoint of an unbalanced translocation from a patient with secondary AML between the markers D7S1925 and D7S1395. This region was recently characterized as a common fragile site in the human genome, named FRA7I. Furthermore, the translocation breakpoint of a t(5;7)(p13;q35) of a patient with therapy-related AML was cloned into a 100 kb sized genomic segment that is located centromeric the CNTNAP2-gene close to the proximal border of the CDS. Conclusions. Our data further indicate the remarkable heterogeneity of deletion and translocation breakpoints on 7q and revealed several hot spot regions that may serve as important starting points for the identification of pathogenetically relevant genes.

**0583**
SERIAL ANALYSIS OF CHROMOSOME ABERRATIONS IN MULTIPLE MYELOMA: HIGH PREVALENCE OF STRUCTURAL ABNORMALITY OF CHROMOSOME 1 DURING DISEASE PROGRESSION

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**Background.** Two major genetic subtypes of multiple myeloma (MM) have been proposed: the hyperdiploid subtype characterized by multiple trisomies and low prevalence of del(13q), and the non-hyperdiploid subtype characterized by IgH translocations and del(13q). Primary IgH translocations have been considered as initiating events in the pathogenesis of MM. The role of del(13q) and other chromosomal abnormalities in disease progression have not been clarified. Aims. To investigated the evolution of chromosomal abnormalities during disease progression. Methods. Analysis of the cytogenetic abnormalities of serial bone marrow samples from MM patients that entered in the clinical cytogenetic database at Erasmus MC. Results. Seventy-five serial samples obtained from 36 patients at diagnosis (26 samples) and during progression of the disease (49 samples) were included in this study. The mean interval between the samples was 26 months (range 6-77). Samples without clonal abnormalities were considered as failures. Using conventional cytogenetic banding techniques, 15/56 (56%) initial and 19/39 (49%) follow-up samples had an abnormal karyotype. The origin of respectively 19 and 37
marker chromosomes could not be identified. Of the remaining 32 samples with t(4;11) we detected 15 with diploid and 17 with hyperdiploid, respectively. Serial studies showed an increased number of chromosomal abnormalities during disease progression. The mean number of aberrations increased from 11 (range 1-38) to 18 (range 2-36). Trisomies of chromosomes 5, 9, 11, 15 and 19 were the most common numerical abnormalities. Monosomy of chromosome 15 was identified in 5/18 initial and 7/19 follow-up samples with the MLPA technique. The chromosomal aberration of chromosome 1 was the most common structural abnormality (25/162) (15%) and were detected in 2/13 initial and 10/19 follow-up samples. Both the short and long arms of chromosome 1 were involved and no-specific locus was predominantly affected. The rearrangement of chromosome 1 consisted in the majority of unbalanced translocations and resulted in the formation of a novel t(1p/1q). Aberrations of chromosomes 8 and 22 were second in frequency (6%) and were detected in 1/13 initial and 9/19 follow-up samples. FISH analysis was performed in 56 samples and showed an abnormality in 15/22 (68%) initial and 25/34 (74%) follow-up samples with probes specific for KB-1 (13q14) and D15S19 (13q14.3) loci and for the centromere regions of chromosome 9 and 11. Del(13q) was observed in 10/22 (45%) initial and 14/34 (41%) follow-up samples. Summary. Cytogenetic abnormalities in multiple myeloma are not random. Disease progression is correlated with increasing complexity of cytogenetic karyotype, which consist mainly of structural aberrations acquired during later stages of the disease. Aberrations of chromosome 1 are common in this myeloma. In particular, unbalanced translocations of 1p/1q have been delineated as genetic event associated with progressive disease and unfavourable prognosis. Del(13q) is not associated with disease progression.

References
patients) could be successfully identified either by RT-PCR or by direct genomic PCR. This controversial data provoked us to investigate "MLL-AF4+/-MLL" leukemia patients in more detail by using a LDI-PCR based method. This allows to identify and characterize chromosomal aberrations of the human MLL gene in an unbiased fashion. 13 individual MLL-AF4+/AF4+/-MLL- leukemia patients out of 76 (t(4;11) leukemia patients were identified (65 were MLL-AF4+/-AF4+/-MLL+ leukemia patients). The 13 MLL-AF4+/AF4+/-MLL- leukemia patients were analyzed for the presence of rearranged genomic MLL sequences. 10 patients displayed a complex rearrangement between chromosome 4 and 11 (and sometimes a third chromosome) that involved at least the MLL, the AF4 and a third partner. Funded by grant 2002.061.I from the Wilhelm Sander Foundation to R.M., T.K. and T.D.

**0587**

**FISH-MLL ABNORMALITIES IN PATIENTS WITH ACUTE MYELOBLASTIC LEUKEMIA AND ASSOCIATION WITH FLT3 AND MLL INTERNAL DUALPLICATION**

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Background. The MLL gene on chromosome 11q23 is frequently involved in haematological malignancies. It is possible to subdivide the MLL abnormalities in two groups: 1) rearrangements, usually as translocations or insertions, and partial tandem duplication (PTD); 2) amplification of the 11q23 region, leading to the presence of multiple copies of the MLL gene, located either intrachromosomally, as der(hsr) and iso11q, or extrachromosomally as dicentrics and numerical abnormalities of chromosome 11. MLL/PTD is the in-frame fusion of a duplicated portion of the MLL gene. Internal tandem duplication (ITD) or mutations have been demonstrated as a activating mechanism also in another oncogene involved in AML, FLT3 gene, which encodes for a tyrosine kinase receptor widely expressed in hematopoietic lineage. The FLT3/ITD is observed in approximately 20% of unselected de novo adult AMLs, with a higher frequency around 30-40% reported for patients with normal cytogenetics. It is associated with poor prognosis in most series. It has been reported that FLT3/ITD is more common in patients with MLL/PTD than in cases with MLL translocations. Recently, a role for coduplication of MLL and FLT3 genes has been suggested in AML as possible marker of a common genotoxic stress. Aim. We investigated the incidence of MLL abnormalities in 207 patients with de novo acute myeloid leukemia, diagnosed following FAB criteria and treated according to the GIMEMA protocols. We used conventional cytogenetics and fluorescent in situ hybridization (FISH) analysis with a MLL probe. The patients were also tested for the presence of an internal duplication of the MLL and FLT3 genes. Materials and Methods. Cytogenetic analysis was performed in 175 cases and showed abnormalities of chromosome 11 in 13 patients (18.7% vs 5.3% of unselected AML). AML and the FISH analysis allows to improve the characterization of MLL involvement when compared with conventional cytogenetics. The incidence of FLT3 alterations is similar in MLL abnormal patients (20%) when compared to the whole AML population (27.4%); on the contrary, MLL/PTD is confirmed to be more frequent in patients with abnormalities of chromosome 11 (18.7% vs 5.3% of unselected AML). The rate of MLL/PTD was superior in FLT3 positive (7.7%) than in FLT3 negative patients (4.4%). In this study the coduplication of FLT3 and MLL/PTD had a low incidence around 2.3% in all cases and did not correlate with cytogenetic MLL abnormalities.

**0588**

**A NEW CRYPTIC MOLECULAR LESION UNDERLIES 6P CHANGES IN SECONDARY ACUTE MYELOID LEUKEMIA/MEYLOIDYSPLASTIC SYNDROME (AML/MDS)**

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Background. Secondary AML/MDS are frequently associated with complex karyotypes involving chromosome 5, 7, 11, 12, 17, 18, and 21. Specific genetic pathways are related to physical and/or chemical toxicants, such as -5/-5q- to alkylating agents or 11q23 and 11p15 changes to topoisoamer II inhibitors. 6p aberrations are cytogenetically heterogeneous and often belong to complex karyotypes with del(5)(q)/-5 and/or del(7)(q)/-7. Aim. Molecular characterization of 6p rearrangements in secondary AML/MDS. Methods. We selected nine patients with secondary AML/MDS and one Fanconi Anemia patient with MDS with a rearrangement on the short arm of chromosome 6. Karyotypes of G-band metaphases were described according to ISCN (1995). Metaphase FISH with a panel of 38 DNA clones for 6p12-p25 bands were performed in all cases. Interphase FISH, CGH and molecular abnormalities of chromosome 6p were detected by interphase FISH, CGH and molecular clones/paints and/or centrometric probes were performed in selected cases. Results. 6p rearrangements were isolated in 4 patients and included in complex karyotypes in 6. Numerical or structural aberration typically associated with therapy-related AML/MDS, i.e. -5/-5q-, -7/7q-, monosomy 18 were respectively found in four, three, and three patients. In three cases full or partial trisomy of the 6p arm was present i(6)(q10) in one case and dup(6)(p) in two patients. The remaining 7 patients showed 6p unbalanced translocations with diverse chromosome partners or unidentified material. In 5 patients with unbalanced translocations, FISH detected cryptic duplications of a genomic region contiguous to the common breakpoint areas, at band p21, while in two patients a low copy gain with five copies of DNA clones mapping at band p21, were present on der(6) and/or inserted in other derivative chromosomes. In all cases a common over-represented 6p21 region was narrowed to a 5.6 megabase DNA segment extending from the TNF gene to ETV7. Two patients did not show 6p21 gain. Conclusion. 6p21 gains, either as duplication/trisomy or low copy gain, emerged as a new recurrent genomic lesion in secondary AML/MDS with 6p abnormalities. Remarkably, they may be cryptic at conventional cytogenetics and underlie different types of chromosome changes. Putative candidate genes, such as the MHC complex, NOTCH-4, BAK, IANSE, ETV7, HMG1Y and FKBP51, map within the common over-represented 6p21 region. In all patients low copy gains occurred in both treatment and environmentally induced AML/MDS as well as in the FA patient, toxic insults and congenital instability appear to share the same genetic pathway. Acknowledgements. BAC clones were kindly provided by Dr. M. Rocchi, University of Bari, Italy. Supported by: CNR-MIUR, FBBR, Associazione Segio Luciani, Fabbrano, and Fondazione Cassa di Risparmio, Perugia, Italy. B.C. is supported by a grant from FIRC (Fondazione Italiana Ricerca sul Cancro).

**0589**

**CYTOGENETIC AND FISH STUDY IN 203 B-CLL PATIENTS**

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Background. The progress in molecular genetic characterization of chronic lymphocytic leukemia (CLL) revealed the prognostic role of IgVH mutational status, of phenotypic changes involving expression of CD38 and ZAP-70, as well as, of chromosomal abnormalities defined by molecular cytogenetic Methods. Interphase fluorescence in situ hybridization (i-FISH) is able to detect the most common chromosomal abnormalities (i.e. -1q, 17p deletions and trisomy 12). Aim. The current study was to determine the chromosomal abnormalities in 203 CLL patients using cytogenetic and molecular cytogenetic methods, and to correlate the molecular cytogenetic findings with disease status (stable versus progressive), with immunoglobulin variable heavy chain (IgVH) mutational pattern, and with other clinical parameters. Methods and patients. 123 males and 80 females (median of age 62 years) were examined by con-
A MOLECULAR CYTOGENETIC STUDY OF MANTLE CELL LYMPHOMA AT DIAGNOSIS AND FOLLOW-UP: EVIDENCE FOR A 'TEMPORALLY ORDERED' CYTOGENETIC EVOLUTION?

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Background. Apart from t(11;14)(q21;q32), MCL is also characterized by other nonrandom cytogenetic findings. These additional aberrations are well studied at diagnosis and believed to represent clonal evolution during lymphomagenesis, but little is known about karyotypic changes during the course of the disease. Methods. The study included 33 patients with MCL. In all cases, an interphase FISH assay was performed at diagnosis. The deletion of 1q14 was the most frequent (43%) chromosomal aberration detected by I-FISH in our cohort of CLL patients. The correlation of molecular cytogenetic results with IgVH mutational pattern and with clinical data were analyzed and will be presented.

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PROGNOSTIC SIGNIFICANCE OF COMPLEX CHROMOSOMAL Rearrangements IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA

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Background. Ph chromosome i.e. translocation t(9;22)(q34;q11) is a specific chromosomal aberration in bone marrow cells of patients in chronic phase (CP) of CML. During progression of the disease from the chronic to the accelerated phase (AP) and/or blast crisis (BC), clonal evolution with non-random secondary numerical and structural aberrations is frequently observed. Complex chromosomal rearrangements (CCR) are rather rare and the significance and frequency of different anomalies are poorly understood. Aims. The aim of our study was a comprehensive analysis of complex chromosomal rearrangements found in bone marrow cells of 22 patients with CML by molecular cytogenetic methods, determination of chromosomes and chromosomal parts which are involved in CCR during progression of the disease and estimation of frequency of non-random changes if they exist. Methods. For the assessment of BCR/ABL fusion gene at the time of diagnosis RT-PCR and/or interphase FISH with locus-specific probe (Abbott-VysisTM) were used (200 ing interphase nuclei analyzed for each case). In some patients further molecular analyses were performed by real-time RT-PCR according to EAC protocol using β-2-microglobuline as a control gene. Multicolor FISH (mFISH) was carried out using the '24XCode' Metasystems 24 color kit (Metasystems TM) to identify precisely complex chromosomal rearrangements in 22 patients. Most of the patients were in the CP at diagnosis. In the course of the disease, clonal evolution with complex chromosomal rearrangements appeared in eight patients who remained in CP, two patients progressed to AP and the rest of them to BC. Results. The majority of the structural changes were unbalanced. Variant Ph translocations (involving chromosomes 9, 22 and one or more other chromosomes) were found in ten patients, the rest of the cohort had a classical Ph translocation associated with additional structural aberrations. The most frequent chromosomes involved in CCR were found to be Nos. 2 (6x), 7 and 17 (5x), 1, 3 and 4 (1x), 5 (1x). Chromosomal regions 1p, 2p, 5q, 7p and 17p were often involved in CCR and the bands repeatedly affected were 17p11.2 (5x) and 7p15 (2x). No one of complex translocation was seen more than once. Conclusions. The results of this study demonstrate the very high instability of the genome of malignant cells at the chromosomal level than was expected on the basis of classical cytogenetic analyses. We also proved that CCR are associated with rather poor prognosis and respond poorly to antikemic treatment. Analysis of CCR by mFISH is important as we believe that such examinations of large cohorts of patients could confirm the significance and non-randomness of this instability and to find out possible recurrent chromosomal aberrations specific for disease progression. Precise determination of breakpoints on chromosomes involved in CCR of bone marrow cells of CML patients can give new dimension to our understanding of genetic mechanisms which can play role in progression of malignant disease.

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TRANSLLOCATION T(9;14)(P13;Q32) IN THREE CASES OF SPLENIC MARGINAL ZONE LYMPHOMA

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Background. Translocation t(9;14)(p13;q32) involving PAX-5 and IgH is an aberration that was first described in lymphoplasmacytic lymphoma (LPL). Nevertheless, new data suggest that t(9;14) is not restricted to a specific morphologic subtype and it is recurrent in other B-cell lymphomas (high-grade and low-grade). Moreover, chromosomal studies in splenic marginal zone lymphoma (SMZL) revealed a high incidence of deletions of 7q and gains of 9q and few incidence of translocations involving 14q32. Reviewing reported cases, only one SMZL patient with a translocation t(9;14)(p13;q32) was described. Aims. The aim of this study was to present the finding of t(9;14)(p13;q32) in SMZL patients (diagnosed by cytology, immunophenotype and his-
DETECTION OF STRUCTURAL ABERRATIONS OF CHROMOSOME 7 IN MYELOID MALIGNANCIES USING COMBINATION OF MOLECULAR CYTOGENETIC TECHNIQUES

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Background. Complete or partial loss of chromosome 7 is a frequent chromosomal aberration in myeloid disorders such as myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML). Allelotypic studies have delineated at least three distinct loci, that are frequently deletet: 7q22, 7q31 and 7q35. It has been hypothesized that there are localized tumor suppressor genes that contribute to the pathogenesis of these disorders. Aims. Using combinations of conventional and molecular cytogenetic techniques we have focused on the analyses of deletions and translocations involving chromosome 7 in bone marrow cells of patients with MDS and AML. Correlation of clinical characteristics, outcome and survival of patients according to cytogenetic findings were evaluated. Methods. Using classical cytogenetic techniques we examined 32 patients with myeloid malignancies (16 MDS, 16 AML), whose bone marrow cells contained 7q deletion or rearrangements of chromosome 7. Fluorescence in situ hybridization (FISH) with locus specific probes for 7q31 region (ABBOTT VYSIS), and 7q22/q35 specific probe (Cibio gene) were used in all patients to confirm the deletion and to prove the breakpoints. Multicolor banding technique (mBAND) for chromosome 7 was carried out in 16 patients for precise mapping of the extent of deletions (XCyte 7 DNA Probe Kit, MetaSystems). Chromosomes involved in complex translocations were identified by multicolor FISH (mFISH) (24XCyte DNA Probe Kit, MetaSystems). Results. By using conventional cytogenetic techniques deletion of 7q was found in 5 patients, in two as a sole aberration, in 27 patients translocation of chromosome 7 was ascertained. According to the results of FISH with locus specific probes for 7q22, 7q31 and 7q35 region and mBAND for chromosome 7 five groups of patients were established: patients with deletion 7q as a sole aberration (2x), patients with deletion 7q and complex karyotype (5x), patients with combined translocation and deletion 7q (19x), patients with combined translocation and deletion 7p (5x) and patients with translocation of chromosome 7 without deletion 7p or 7q (5x). Deletions of all three FISH screened regions on the long arms were the most frequent, the breakpoints were heterogeneous and varied among patients. On the short arms of chromosome 7 region 7p13.2p15.2 was the common deleted segment. Complex karyotype was confirmed by mFISH in 29 patients. Most of the deletions in patients with complex karyotype were cryptic, not detectable using conventional cytogenetic techniques. Summary. Aberrations of chromosome 7 are associated with a poor prognosis, increased risk of infection, rapidly progressive disease and poor response to treatment. Survival time was short in our cohort of patients (median 7 months), 25 patients died. Systematic molecular cytogenetics studies reveal cryptic rearrangements and provide novel information about genes possibly involved in these events.

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T(5;12)(Q23-31;P13) WITH ETV6-ACSL6 GENE FUSION IN POLYCYTHEMIA VERA

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Background. Myeloproliferative disorders (MPD) are chronic clonal proliferations of haematopoietic progenitors. Typical MPDs include chronic myeloid leukaemia (CML), polycythemia vera (PV), essential thrombocytopenia (ET) and idiopathic myelofibrosis (IF). Oncogenic alterations identified so far in MPDs target tyrosine kinases, result from chromosomal translocations or gene mutations and lead to constitutive activation of survival and proliferation pathways. Reciprocal translocations lead to gene fusion and production of chimeric proteins such as BCR-ABL, ETV6-PDGFRB or PCM1-JAK2. Point mutations of the JAK2 kinase occur in almost all PV, and in around half of ET and IF. JAK2 functions downstream of membrane receptors, including cytokine receptors such as IL3 receptors. Overproduction of IL3 has been reported in atypical CML following rearrangements of the IL3 gene upstream region in cells from patients with t(5;12)(q23-31;p13) translocation and ETV6-ACSL6 fusion. Aims. We report here two cases of t(5;12)(q23-31;p13) translocation with ETV6-ACSL6 rearrangement in PV patients. Methods and results. Cytogenetic analysis with R-banding technique on BM cells detected a t(5;12)(q23-31;p13) translocation. We demonstrated the involvement of ETV6 and the 5' region of ACSL6 (previously ACS2) in the translocation by using dual-color fluorescence in situ hybridization (FISH) on metaphases of MPD cells from one patient, using labeled-BAC clones. The 5' breakpoint was located in the 5' region of ACSL6. We detected a t(5;12) in almost all PV, and in around half of ET and IF. JAK2 functions downstream of membrane receptors, including cytokine receptors such as IL3 receptors. Overproduction of IL3 has been reported in atypical CML following rearrangements of the IL3 gene upstream region in cells from patients with t(5;12)(q23-31;p13) translocation and ETV6-ACSL6 fusion. In conclusion, we have described a genomic alteration that had never been described before in PV patients. As suggested the result of the rearrangement could be an upregulation of the IL3 gene. Due to the orientation of the rearranged genes on the der(12), it is probable that the cause of this upregulation is a chromatin conformation change rather than an ETV6 promoter effect. In one of the patient samples, we found no evidence of a JAK2 mutation in the same clone but not detected by our DNA analysis although its measured sensitivity was below this range. In this case, the genomic events such as a t(5;12) rearrangement may account for few JAK2-negative PF.

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ADDITIONAL CHROMOSOMAL ABNORMALITIES IN PH-POSITIVE CML DE NOVO: A MULTICENTER STUDY

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Chronic myeloid leukemia (CML) is a clonal disorder of multipotent haematopoietic cells associated with specific cytogenetic changes involving a translocation t(9;22)(q34;q11) resulting in Ph chromosome occurrence. Cytogenetic investigations revealed that Ph chromosome appeared most often as a sole karyotype aberration during the chronic phase, whereas additional changes frequently accompanied or preceded a transformation to the advanced stages of CML (50-80%). However, the additional cytogenetic changes are found in 10-20% of CML patients at diagnosis and their prognostic impact is still difficult to assess unequivocally. Cytogenetic study as a part of Polish national program of Development of standard operating procedures for the diagnosis and follow up of treatment of CML in 2005 was performed to evaluate the appearance and the frequency of additional cytogenetic changes in patients with de novo CML. Material and Methods. A total number of 206 newly diagnosed Ph-positive CML patients investigated in 6 cytogenetic centers formed the subjects of this study. All cases were analyzed by routine cytogenetic techniques on unstimulated and/ or stimulated bone marrow samples according to standard protocols. Karyotypes were described in accordance to the ISCN1995. Additionally, nearly all cases underwent FISH, RT-PCR, and RQ-PCR analysis for BCR/ABL. Results. 40 of 206 patients (19.42%) showed aberrations different from simple t(9;22). Constitutional changes [inv(9)(p13q13)] were found only in 2 patients. In the remaining patients, karyotypes showed rearrangements related to leukemia. Three-way Ph translocations were observed in 13 cases (32.5%), involving nonrandom chromosome bands, such as 1q21, 2p13, 3p21, 3q21, 10q25, 10q25, 15p11, and 17q24. Other structural changes, not related to Ph translocation, accounted for 30% of cases (12/40): add(1)(p36), t(1;19)(q32;p13), t(1;17)(p32;p13), add(2)(qter), t(4;22)(p16;q11), t(4;22)(q31;q13), del(6)(q21), t(7;17)(q32;q25), inv(16)(p11.2q24), del(17)(p15), add(21)(p11), add(15)(p15). Numerical aberrations were also observed in 30% of cases (12/40). Beside the most common trisomy/tetrasomy 8 and +Ph, the aberrations: +X, -Y, +15, +17, +19, -21, +21, were disclosed. Few unidentified markers were also revealed. Conclusions. Among described aberrations, we found significant part of nonrandom cytogenetic changes described previously, but some of them, to our knowledge, are described for the first time in relation with CML. For all patients with additional aberrations further observations and at least 1 year follow-up are needed to evaluate their prognostic significance based on clinical stage, prognostic scores and response to treatment.

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FUNCTIONAL ANALYSIS OF CANDIDATE GENES LOCALISED IN 13q14.3, A REGION COMMONLY AFFECTED IN B-CLL
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Background. Genomic material from chromosomal band 13q14.3 is lost in a variety of neoplasms. Thus, a tumor suppressor mechanism distinct from the RB1 gene has been postulated in this region. In B-cell Chronic Lymphocytic Leukemia (B-CLL), the most common leukemia in the Western world, deletion within chromosomal band 13q14.3 is the most frequent genomic imbalance. However, the pathomechanism in the critical region has not yet been defined. Characterisation of the function of genes in the critical region will allow identification of the most likely tumor suppressor candidates and their role in tumor pathomechanism. Therefore, we analysed the function of different candidate genes localised in 13q14.5. Aim. The aim of this project is functional analysis of candidate genes localised in 13q14 such as RFP2, C13orf1, DLEU2/LEU2/BCSMUN/DLB2 and KPNAS. To this end, expression levels of those genes were modulated by knock down and overexpression followed by subsequent analysis of transcriptome changes. Methods. Gene expression was modulated by overexpression using cDNA plasmids and knock down using RNAi. A combined lipofection and electroporation technology was used in order to obtain sufficiently high transfection efficiency in cell lines with loss of 13q14.5. RNA was isolated from cells after 4, 7 and 12 hours to check genome wide gene expression levels via oligonucleotide arrays. In a second strategy, we used microRNA technology to knock down RFP2, C13orf1, DLEU2 and KPNAS in human embryonic kidney cells (HEK-293) and HELA cells. RNA was isolated after 48h to identify effects in downstream target genes via expression profiling. Differentially expressed genes of both strategies were verified using Real-Time-PCR. Results. To analyse phenotypic changes in transfected cells, a significant overexpression or knock down of the transduced genes was essential. For RFP2 and C13orf1, we could achieve an overexpression of over 90 fold compared to the transfection of empty vector. Overexpression of DLEU2 was only possible up to 12 fold. In HEK and HELA cells, the knock down of C13orf1 and KPNAS was over 70%. For RFP2, a 60% knock down could be achieved and for DLEU2, the knock down was between 40 and 50%. Downregulation of KPNAS by RNAi was also shown on protein level. Using RNAs in mammalian cells specificity of knock down has to be shown. There exists an interferon response mechanism in case double stranded RNA is injected into the cell for example by a virus. To be sure not to induce the interferon response in our siRNA transfected cells, we checked expression level of a marker gene for interferon response (OAS1). Using both strategic approaches, we could identify a number of gene products which are affected by modulation of expression of candidate genes of B-CLL. Summary. Candidate genes of B-CLL were modulated in their expression levels and phenotypic effects were analysed by expression profiling genome-wide. Our results suggest involvement of different signalling pathways in the pathomechanism residing in 13q14.

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VALUE OF PROTEOMIC SCREENING FOR PREDICTION OF GRAFT VERSUS HOST DISEASE AFTER ALLOGENIC HEMATOPOIETIC STEM CELL TRANSPLANTATION
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Background. We have recently published a polypeptide pattern specific for the early diagnosis of acute GvHD (aGvHD), based on the application of capillary electrophoresis (CE) and mass spectrometry (MS). Aims. Here we report the application of an aGvHD-specific pattern to prospectively and blinded collected samples from 76 patients (53 AML, 10 sAML, 13 ALL, 2 MDS, 6 PCT, 5 SAA, 3 CLL, 2 MFE; 2 Hodgkin Lymphoma, 1 CML, 1 NHL[mhh]). Fifty patients were transplanted from matched unrelated donors (MUD), 27 received stem cells from matched related donors (MRD), 2 from haplo-identical donors. In the majority of the patients the GvHD prophylaxis was methotrexat (59) or mycophenolate(24) and cyclosporin A. 2 patients received T-cell depleted grafts and 4 received steroids instead of MTX. Methods. Urine samples were collected on ice prior to conditioning, weekly until discharge from the ward and monthly thereafter. Immediate freezing of the samples avoids degradation of the proteins/peptides. After thawing and removal of confounding materials, like salts, or molecules larger than 30 kDa, the samples were loaded onto the CE, separated according to their charge and, after ionization, directly analyzed in an electrospray ionization time of flight (ESI-TOF)MS. This lead to the detection of 500 up to 2500 peptides and proteins in individual samples. Results. The polypeptide pattern specific for the detection of acute GvHD grade II or greater were applied to the data from the prospectively and blinded collected samples. A total of 760 samples were evaluated using this set up. In general between 4 and 10 samples were collected and screened after HSCT, whereas the control groups (other diseases) contain only 1 sample per patient. Taken together, 800 samples from patients after HSCT have been prospectively evaluated. The sensitivity was 92.5% with a positive prediction value of development of aGvHD of 83% and the specificity was 94% with a negative prediction value of more than 98%. Conclusion. We have shown that the application of a peptide pattern, consisting of several differentially excr eted peptides allows prediction of the development of aGvHD. Especially patients developing steroid resistant aGvHD show the aGvHD-specific changes very early (about 10 days) prior to clinical symptoms. Taken together a therapeutic strategy using the aGvHD pattern as guidance for an early start of immunosuppression seems justified.
causes and role of deregulated miRNA expression in malignancy are emerging. However, GBD mutations do not seem to be a presenting feature of GBD mutations of Wiskott-Aldrich syndrome protein (WASP). Male/female ratio was 1.35. There were two brothers with monosomy 7. There were 173 adults (median age 65y, range 21-89y) and 34 children (<20y, median age 12y, range 2-20y). 56 Cases had monosomy 7. Male/female ratio was 1.35. There were two brothers with monosomy 7, one with AML M5 and the other with secondary AML all after MDS. Exons 7-10, encoding the GTPase Binding Domain (residues 230-381), were successfully amplified for mutations using HPLC. DNA from case I294T (Genet 2001, 27, 313) was used as positive control. Results. We observed 2 myeloid malignancies among the 5 affected members in this family. Patient I13 developed a myelodysplastic syndrome (MDS-RAEB) at age 65, while under hematopoietic growth factor support. He died after 6 months of disease progression. Patient II3 developed a myelodysplastic syndrome (MDS-RAEB) at age 65, while under hematopoietic growth factor support. He died after 6 months of disease progression. We observed 2 myeloid malignancies among the 5 affected members in this family. Patient I13 developed a myelodysplastic syndrome (MDS-RAEB) at age 65, while under hematopoietic growth factor support. He died after 6 months of disease progression. Patient II3 developed a myelodysplastic syndrome (MDS-RAEB) at age 65, while under hematopoietic growth factor support. He died after 6 months of disease progression.
ment analyses showed a significant down-regulation of genes involved in DNA metabolism and DNA repair. First results from our ongoing analysis of in vivo VPA treated samples are encouraging as we e.g. also see an induction of CDKN1A expression. However, the picture observed is less homogenous as concomitant administration of ICE, as well as other factors like e.g. VPA serum levels might substantially influence the in vivo VPA response. In addition to the expression profiles of MKs and novel transcripts and to investigate the expression of these genes in other haematopoietic lineages. The presence of selected transmembrane proteins on platelets and MKs was confirmed using murine anti-sera generated against recombinant, E. coli expressed protein. Gene silencing experiments in Zebrafish and human MKs are in progress to determine the biological role of these novel proteins.

**0603**

**GENOMIC APPROACH TO UNDERSTAND THE MECHANISMS INVOLVED IN BCR-ABL-MEDIATED RESISTANCE TO APOPTOSIS**


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**Background.** Bcr-Abl is an oncoprotein involved in malignant transformation and resistance to apoptosis, processes that are not fully understood. *Aims*. To investigate the impact of enforced expression of Bcr-Abl on global gene expression and apoptotic machinery. *Methods*. Ectopic expression of Bcr-Abl was obtained using retroviral vectors. Apoptosis was evaluated against different stimuli by flow cytometry (annexinV/PI and DAPI). The percentage of apoptosis of oncogenes was examined by RT-PCR and Western-blot. Global gene expression of wild-type and Bcr-Abl+ cells was performed with oligonucleotide microarrays. Differentially expressed genes were identified based on log-ratios consistently above/below intensity-dependent cutoff curves obtained by applying HTseq method to self-self data. *Results*. Microarray analysis of Bcr-Abl transfectants identified 465 common genes that were overexpressed and 70 underexpressed in cells that became resistant to apoptosis. The upregulated genes are mainly related to cell motility, communication, growth, death, signal transduction and metabolism. Among them, genes involved in apoptosis and immune system regulation such as fasn, caspase-9, nfat, and p53 were found. Most of the underexpressed genes are related to signal transduction pathways (tyrosb, calcineurin A β, and traf-3). Bcr-Abl expression conferred resistance to death-inducers in HL-60 and HeLa cells, but not in SKW6.4 cells. Protein levels of Bcr-Abl, Mcl-1 and Flip were higher in HL-60/Bcr-Abl than in HL-60. These effects were observed in HeLa.Bcr-Abl and SKW6.4.Bcr-Abl. In contrast, pro-apoptotic protein Bid was reduced in HL-60/Bcr-Abl and SKW6.4/Bcr-Abl. *Conclusions*. In sum, ectopic expression of Bcr-Abl is capable of protecting HL-60 and HeLa, but not SKW6.4 cells from apoptosis. 

**0604**

**NOVEL TRANSMEMBRANE MEGAKARYOCYTE PROTEINS IDENTIFIED BY COMPARATIVE GENE EXPRESSION PROFILING OF IN VITRO DIFFERENTIATED HUMAN MEGAKARYOCYTES AND ERYTHROBLASTS**

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We have used cDNA microarrays to investigate the transcriptomes of megakaryocytes (MKs) and erythroblasts (EBs) to identify genes that are differentially expressed between the two cell types. MKs and EBs have a common, bipotent progenitor and we hypothesised that the subset of genes that are differentially expressed between them might contain novel ‘lineage specific’ elements. CD34+ Haematopoietic Stem Cells (HSCs) isolated from Umbilical Cord Blood were purified by magnetic cell separation. For differentiation into MKs, HSCs were cultured for 7 days in serum-free medium containing thrombopoietin (TPO) and IL-1β. For differentiation into EBs, HSCs were cultured for 10 days with Erythropoietin (EPO), IL-3 and Stem Cell Factor (SCF). Differenctiated cells were purified by Fluorescence Activated Cell Sorting (FACS) to greater than 95% purity prior to RNA isolation. RNA was amplified and labelled using the SMART Template-Switching PCR protocol and the gene expression was then directly compared using cDNA microarrays containing 15,000 features (Sanger Herv2.1.1). Twenty hybrids were performed, representing five biologically paired comparisons, each with four technical replicates. Statistical analysis of this data identified 685 features that were upregulated in MKs (>2-fold, p<0.05), and 219 features in EBs. Known lineage specific markers, such as ITGA2B (CD41), GP9 (CD42a) and GP1B (CD42b), were upregulated in MKs relative to EBs as expected. In addition, a number of novel transmembrane proteins were also identified as upregulated in MKs. RT-PCR was used to validate the differential expression of both known and novel transcripts and to investigate the expression of these genes in other haematopoietic lineages. The aim of this study is to identify, in an unbiased way, hypermethylated and transcriptionally inactive sites in the genome of the OCI/AML2 cell line using microarray technology. *Aims*. The aim of this study is to identify, in an unbiased way, hypermethylated and transcriptionally inactive sites in the genome of the OCI/AML2 cell line using microarray technology. *Methods*. We used an anti-5-methyl-cytosine antibody to pull down methylated sequences from sonicated genomic DNA from the OCI/AML2 cell line. The immunoprecipitated DNA was labeled and hybridized onto the University Health Network (UHN) 12K CpG array (www.microarry.ca) and after visual inspection positive clones were identified. These clones were then intersected with predicted promoters, CpG islands and flat exons (First EF) using the UCSC table browser intersection function. After this filtering step the clones were mapped to the chromosome ideogram using KaryoView (www.ensembl.org). Expression profiling of the same cell line was done using the Affymetrix U133A array. Under-expressed genes were also mapped to the chromosome ideogram using KaryoView. Statistical analysis of overrepresented cytogenetic bands was done using DAVID 2.1. *Results*. After careful analysis and filtering of uninformative clones we found a good correlation between hypermethylated regions of the genome and regions with relative transcriptional inactivity. Some previously reported genomic loci were also found using this technique; such as the 11q22-25 tumor suppressor locus that has been found to be methylated in nasopharyngeal carcinoma and deleted in CLL. Other sites identified using this screen include the 1q13 locus, that has been reported to be methylated in acute lymphoblastic leukemia and a common site for loss of heterozygosity in prostate cancer and the 6p21 locus that has been found to be hypermethylated in normal ageing. *Conclusion*. Global methylation analysis using CpG microarray technology coupled with gene expression data together with bioinformatics has the potential to detect new tumor suppressor regions in the genome of cancer cells. The use of this technique will further the understanding of the epigenetic processes involved in abnormal gene regulation in AML. This technique may also prove to be useful in the classification of the disease.
**0606**

**EXPRESSION SIGNATURE OF GENES ASSOCIATED WITH TELOMERE-TELOMERASE COMPLEX IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES AND ACUTE LEUKEMIA: TEPI GENE IS SURPRISINGLY UPREGULATED IN PROGRESSION OF MDS AND IN LEUKEMIC CELLS**

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**Background.** Knowledge of dynamics of telomere-telomerase complex brings important sign into molecular background of leukemogenesis. Misbalance initiated by erosion of telomeres may affect also expression level of genes implicated in regulation of telomere length and telomerase activity. Thus, data on expression profiles of associated genes: hTERT encoding catalytic sub-unit of telomerase, the tankyrase (TNKS), TRF1 (Telomeric Repeat binding Factor 1), POT1 (Protection Of Telomeres 1), TEPI encoding telomere associated protein, and myc may be valuable from the viewpoint of disease prognosis and monitoring of therapy effectiveness. **Aims.** To ascertain expression variations of genes involved in regulation of telomere-telomerase complex in patients with myelodysplastic syndromes (MDS), acute myelogenous leukemia (AML) from MDS, and primary untreated AML with the aim to evaluate their significance as prognostic factors of MDS evolution towards overt leukemia and markers of leukemic cells. **Methods.** The study was done in mono-nuclear bone marrow (BM) or peripheral blood (PB) samples from 42 patients with MDS, AML from MDS and untreated primary AML divided into subgroups according to the FAB criteria. Mono-nuclear cells from 16 healthy BM or PB progenitor cells healthy donors served as normal controls. RNA was extracted using modified method of Chomczynski. Relative expressions of hTERT, TNKS, TRF1, POT1, TEPI, and myc RNA were assayed by real-time RTPCR with specific Taq-Man probes in RotorGene 3000A (Corbett Research) in comparison to expression of the housekeeping gene. Results. The ratio higher than mean + 2 s.d. of healthy controls were postulated as cases with positive gene expression. Expression signatures were discussed together with telomere length, telomerase activity and clinical features: proportion of blast cells, results of the DFS analysis and also with individual patients risk score established for MDS according to the Internation-al Prognostic Scoring System (IPSS). **Results.** Notable increase of expression of hTERT, TEPI, and POT1 genes was observed in patients with advanced form of MDS (RAEB and RAEB-t) in contrast to insignificant changes of telomerase activity representing a later event in misbalance of telomere-telomerase complex. Significant correlation between individual values of POT1 gene expression and telomerase activity confirmed in MDS and AML patients (p = 0.0079) supports role of the POT1 gene as positive molecular regulator of telomerase. On the other hand, no relationships were found between POT1 expression and the IPSS risk score of MDS patients on one side and portion of blast cells in BM/PB both in MDS and AML on the other side. **Summary/Conclusion.** We showed that hTERT and POT1 genes up-regulated already in early forms of MDS and its expression has increasing trend with disease progression. Significantly increased expression of these genes is also feature of mononuclear BM/PB cells of majority of patients at diagnosis of primary AML. These observations predestine POT1 and hTERT genes at least as additional prognostic factors of MDS and molecular markers of AML. High TEPI expression in patients with advanced forms of MDS and AML indicates on its more active role in signaling of telomere-telomerase complex as it has been supposed. **Supported by grant MHCR 0002373601.**

**0607**

**TEN NOVEL MUTATIONS IN THE HMBS GENE RESPONSIBLE FOR ACUTE INTERMITTENT PORPHYRIA**

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**Background.** Acute intermittent porphyria (AIP) is an autosomal dominant disorder caused by a partial deficiency of hydroxymethylbilane synthase (HMBS), the third enzyme in the heme biosynthetic pathway. Clinical features of the disease are intermittent attacks of neurological dysfunctions and pain. In about 75% of patients attacks are precipitated by stress and alcohol consumption. Most of the affected individuals remain asymptomatic throughout their life but 10-20% presenting severe acute attacks. Diagnosis of AIP is often difficult if based on urinary overproduction of porphyrin precursors ALA and PBG only. The erythrocyte HMBS activity is not extensively available and not always informative because of the overlap between the normal and carriers range. The molecular analysis of HMBS gene represents the most reliable diagnostic tool for AIP. The human HMBS gene maps to chromosome 11q24.1-q24.2 with a total of 15 exons. Two distinct promoters direct housekeeping and erythroid specific mRNAs by alternative splicing. So far, more than 210 different mutations have been identified and worldwide in the HMBS gene as responsible for AIP, showing a high genetic heterogeneity. Most of the reported mutations have been detected only in single families, however a prevalence of specific mutations in different geographic areas has been reported. Only preliminary data are available for the Italian population. **Aims and Patients.** In this study we searched for molecular defects in HMBS gene, in order to detect the most common HMBS mutations in Italian subjects affected by AIP. We investigated twelve unrelated patients and their relatives. The diagnosis was based on clinical manifestations, elevated urinary excretion and reduced erythrocyte HMBS activity. **Methods.** The promoters, the entire coding region and the intron-exon boundaries of HMBS gene has been amplified by polymerase chain reaction and submitted to direct automated sequencing. Restriction fragment length polymorphisms, poly-acrylamide gel electrophoresis and XL PCR were performed to confirm the presence of putative mutations. **Results.** Twelve different molecular defects in HMBS gene have been identified. Two novel mutations (77 G>A and 962 A>G) have been reported and ten mutations are new findings: five deletion, one insertion, one splicing defect, one nonsense and two missense. The 447-467del12bp causes the loss in frame of seven aminoacids in the exon 9 and the 13890bp deletion causes the loss of the entire HMBS gene. The 181delG, the 418-419delAA, the 468-470delAA and 852insC mutations cause frameshift leading to a premature stop codon at aminoacid 914; two missense mutation (2427T>C and 1075 G>A) in exon 6 and 15 result in a Leu81Pro and Asp599Asn amino acid substitution respectively. **Summary.** These results allowed the identification of ten novel HMBS mutations. In a previous work, we have identified other 11 new molecular defects for a total of 21 new different mutations restricted to the Italian population. This study confirmed the high heterogeneity of molecular abnormalities responsible for AIP phenotype and the presence of clusters of mutations in particular geographic areas.

**0608**

**ASSOCIATION OF HUMAN PLATELET ALLOANTIGENS 1, HP A2, HPA, 3 HP A4, AND HPA5 ALLELES AND GENOTYPES WITH SICKLE CELL ANEMIA**

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**Background.** Insofar as sickle cell anaemia (SCA) was described as a hereditary state where occultive vascular complications (OVC) and progression to stroke are frequently seen, polymorphisms of human platelet alloantigens (HP) were reported as risk factors for several vascular anomalies, including stroke. With the exception of a lone report documenting association of HPA-5b with SCA OVC, studies on potential association of HPA1 through HP A5 with SCA are lacking. This study investigated the prevalence of HPA1, HPA2, HPA3, HPA4, and HPA5 alleles and genotypes among Bahraini SCA patients and control subjects. Linkage disequilibrium analysis will be used to investigate the disease association of the these polymorphisms. **Method.** This was a case control study. Study subjects comprised 185 SCA patients (mean age 15.6±9.8) and 157 healthy controls (mean age 27.8±15.1); all were Bahraini nationals. Mutation analysis was assessed by PCR-SSP analysis. Statistical analysis was performed on SPSS v. 13.0 statistics software, significance being set at p < 0.05. **Results.** The distribution of HPA2 (p = 0.225) and HPA4 (p = 0.075) genotypes were comparable between SCA patients and controls. In contrast, higher frequencies of HPA1a (p < 0.001), HPA5a (p = 0.007) were found among controls, while HPA3b (p = 0.034) and HPA5a (p < 0.001) alleles were more frequent in patients. Whereas HPA 3a/3a (p = 0.063; RR = 0.463) and HPA 5b/5b (p < 0.001; RR = 1.82) were more prevalent among controls, HPA 1b/1b (p < 0.001; RR = 19.958), HPA 5b/5b (p = 0.042; RR = 1.784), and HPA 5a/5b (p < 0.001; RR = 3.078) were significantly higher among SCA patients. Significant linkage disequilibrium were noted between HPA alleles, with the strongest occurring between HPA1b and HPA5a (p = 0.119; p < 0.001). **Summary/Conclusion.** Differential association of HPA polymorphism with
SCA was noted among Bahraini patients, with HPA1, HPA3, and HPA5 representing genetic risk factors of SCA. In view of the reported link between HPA polymorphism and OVC, our results serve a diagnostic/prognostic role in identifying SCA patients with possible OVC complications, as well in the development of future therapeutic regimen.

0609
GENOTYPE AND CLONAL EVOLUTION IN CHILDHOOD ACUTE LEUKEMIA CASES
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Background. Leukemia is the phenotypic result of multiple events, which can accumulate in a pre-leukemic clone and whose origin can be either pre-natal or post-natal in different leukemia subtypes. The understanding of consequential events is important for the comprehension of the pathogenesis of pediatric acute leukemia. The aim of our study was to search for hidden genetic aberrations by detecting genome-wide loss of heterozygosity (LOH) and genes copy number variation (CNV) with single nucleotide polymorphism (SNP) arrays. Here we report the dissection of genotype and clonal evolution in two cases of childhood leukemia, a twin pair affected by TEL-AML1 positive ALL and a FLT3-ITD positive AML patient, as a paradigm for describing different mechanisms of clonal evolution. Results: the TEL-AML1 positive monozygotic twins with concordant ALL were classified as pre-B ALL and common-ALL. They shared one common Ig/TcR rearrangement amongst others. Both the diagnosis and remission samples analyzed by GeneChip Mapping 10K SNP array showed LOH at the 2q13-14.3 region in both twins, while deletion of the normal TEL allele was only found in twin 2. LOH was not associated with CNV, implying a recombination event resulting in uniparental isodisomy (UPD). Further analyses are necessary to understand the functional implications of this chromosomal abnormality. One hypothesis could be that the twins were born with a genetic predisposition to develop leukemia, along with the translocation. Additional events were then responsible for the overt leukemia. UPD of this region has not been reported in other tumors or in remission samples of leukemia. Moreover, this is the first report on constitutional UPD in leukemia patients. We also studied clonal evolution in a FLT3-ITD positive childhood AML patient, who experienced two relapses and for whom we had the availability of the cord blood (CB) sample. The patient was diagnosed at 6 years of age with AML-M1, a normal karyotype and FLT3-ITD mutation. The same FLT3-ITD clone re-emerged at relapse, three months after auto-BMT. The DNA from CB was negative for the FLT3-ITD RQ-PCR, consistent with the well-established hypothesis that FLT3-ITD mutations represent post-natal events. The GeneChip Mapping 10K SNP array analysis on DNA from the first relapse showed deletion of the long arm of chromosome 9, and LOH on the whole chromosome 13 not associated with CNV. This latter is consistent with UPD, so either non-disjunction or somatic recombination has led to the homozygosity of FLT3-ITD at 13q14. This is emerging as a frequent event of disease progression, subsequent to FLT3-ITD heterozygous mutation. 10K SNP array analysis did not reveal LOH or copy number changes in the diagnostic and in CB samples. Other methods must be applied to find the primary event(s) giving rise to leukemia in association with FLT3-ITD mutation. Conclusions: despite the heterogeneity of the cases presented here, this study shows that genome-wide LOH analysis by SNP arrays represents a powerful tool to unravel cryptic genetic aberrations and to better understand the genetic events cooperating in clonal evolution.

0610
IDENTIFICATION OF NOVEL THERAPEUTIC TARGETS IN ACUTE MYELOID LEUKAEMIA USING A PHOSPHOPROTEOMIC STRATEGY
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Background. Pharmacological inhibition of the dysregulated Bcr-abl tyrosine kinase has emerged as an effective therapeutic strategy in chronic myeloid leukaemia. Although accumulating evidence demonstrates the importance of the constitutive activation of signalling pathways in acute myeloid leukaemia (AML), the development of targeted therapies has been compromised by our limited understanding of the identity of the dysregulated tyrosine kinases in AML. Aims: Reasoning that since protein tyrosine phosphorylation is an important mechanism mediating the transduction of proliferative and survival signals we have utilised a phosphoproteomic strategy to identify dysregulated phosphoproteins in myeloblasts from patients with AML. Methods: Using an anti-phosphotyrosine antibody we have immunoprecipitated proteins from AML blasts, separated proteins by SDS PAGE and identified proteins within distinct bands by mass spectrometry. Results: In primary AML blasts have been compared with CD34+ progenitors from GCSF mobilised normal donors. The methodology was validated using validated-stimulated HL60 cells. Results: 10 patients with a median age of 51 (range 16-90) were studied. 6 patients had a normal karyotype, one good risk cytogenetics and three adverse risk cytogenetics. Mutations in the flt-3 tyrosine kinase gene were present in four patients. Myeloblasts from every patient demonstrated phosphorylation of MAP kinase (MAPK) implying activation of the ras-MAPK cascade. In contrast CD34+ cells isolated from normal donors demonstrated weak or no MAPK phosphorylation. Since each patient demonstrated MAPK phosphorylation irrespective of flt-3 status we next examined their phosphotyrosine proteome. This identified a number of phosphorylated and non-phosphorylated proteins in signalling complexes in anti-phosphotyrosine immunoprecipitated present in AML blasts but not normal CD34+ progenitors. These included receptors (ephrin type A-3, interleukin-13), signalling intermediates (TAP1, RASA1, LARG, cortactin, CD-2 associated protein) and transcription factors (ELK-1, HFK-1). The phosphorylation of a number of the identified proteins was confirmed immunochemically. These proteins identify three functionally distinct groups in AML blasts that were not detected in CD34+ progenitors: (i) intermediates in PtdIns-3-kinase-mediated signalling presumably suppressing apoptosis (ii) intermediates of a tyrosine kinase-mediated ras-MAPK signalling proliferative cascade and (iii) regulators of the actin cytoskeleton and thus cell movement. This strategy also identified a novel pattern of phosphorylation of the S-HT5A receptor in AML blasts raising the possibility that this protein plays a role in the pathogenesis of AML. Summary: Adoption of a phosphoproteomic methodology has identified novel phosphoproteins in AML which require further validation. Intriguingly our data also point to a common intracellular signalling pathway (ras-MAPK) in AML. These observations provide information for the rational development of targeted therapies in AML.

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Non-Hodgkin's Lymphoma - Experimental

0612
GENE EXPRESSION PROFILING OF PRIMARY HODGKIN/REED-STERNBERG CELLS AND THEIR RELATIONSHIP WITH PRIMARY MEDIASTINAL B-CELL LYMPHOMA
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Background. Classical Hodgkin lymphoma (cHL) and PMBCL share some similarities in terms of clinical presentation, histological and immunophenotypic picture, and genetic and pathogenetic features. So far, genome-wide expression profiling studies have compared whole biopsies of PMBCL and other diffuse large B-cell lymphomas (DLCLs) to cHL cell lines, owing to the rarity of primary HRS cells in lymph nodes involved by cHL. These studies reported for PMBCL a partial molecular overlap with cHL cell lines, which was more pronounced than that with other DLCLs. However, cultured HRS cells most likely do not reflect primary HRS cells in all their important features, as they were derived from patients with end-stage disease and from sites (e.g., pleural effusions, peripheral blood) which are very rarely involved by cHL and in which the dependence on the prominent inflammatory background typically surrounding primary HRS cells in the lymph node is lost. Aims: to investigate the genome-wide expression profile of primary HRS cells and its relationship with that of other lymphomas (including PMBCL) and of normal peripheral B-cell subsets. Methods. ~1000-2000 HRS cells are laser-microdissected from H&E-stained frozen sections of cHL samples. After two rounds of in vitro linear amplification, RNA is hybridized to Affymetrix HG-U133 Plus 2.0 chips (interrogating ~54000 probe sets corresponding to ~30.000 genes). Expression profiles are also generated from similar cell numbers of: i) neoplastic cells FACs-sorted from HL cell lines or microdissected from various non-Hodgkin lymphomas, including PMBCL, and from lymphocyte-predominant HL (LPHL) cases; and ii) normal mature B-cell subsets (plasma cells and naive, memory and germinal center B cells) that are MACS/FACS-sorted from primary cases. The latter further split in two sub-branches: one with PMBCLs, Burkitt lymphomas, follicular lymphomas (each of the three forming its own sub-cluster) and DLCLs, and the other branch mainly comprising HLs (with both cHLs and LPHLs tending to form discrete sub-clusters) and T-cell rich B-cell lymphomas. A supervised comparison of primary HRS with HL cell lines shows a highly differential expression (≥4fold change) of ~1200 genes, includ-
PERIFOSINE, AN ORAL BIOACTIVE NOVEL AKT INHIBITOR, INDUCES ANTITUMOR ACTIVITY IN WALDENSTROM MACROGLOBULINEMIA


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Background. Waldenström's macroglobulinemia (WM) is an incurable low-grade lymphoplasmacytic lymphoma. The PI3K/AKT pathway is a critical regulator of cell survival by stimulating cell proliferation and inhibiting apoptosis. Our previous studies using proteomic analysis have demonstrated upregulation of members of the PI3K/AKT pathway in WM. The new AKT inhibitor, perifosine (NSC 639966, Keryx Biopharmaceuticals, NY) has demonstrated activity in other B-cell malignancies. Aims. We hypothesized that the AKT inhibitor, perifosine will induce cytotoxicity in WM. Methods. WM cell lines (BCWM1 and WSU-WM) and IgM secreting low-grade lymphoma cell lines (MEK1, RL) were used. WM primary malignant cells were obtained from patients after informed consent. Inhibition of proliferation was measured using the MTT proliferation assay. DNA synthesis was measured using the thymidine uptake assay. Apoptosis was determined using Apo2.7 flow cytometry analysis (Beckman Coulter Inc., CA). Bone marrow (BM) stromal cells confer growth and resistance to conventional treatments. We therefore, tested the effect of perifosine on WM cells co-cultured with BM stromal cells. Cell cycle analysis was performed using flow cytometry with PI staining (Molecular Probes, Oregon). IgM secretion was tested using ELISA assay (Immuno-tek, NY). Immunoblotting for pAKT, pERK1/2 and pJNK was performed at 6 hrs of treatment. A two-sided t-test was used to determine differences in response. Results. Perifosine induced significant cytotoxicity and inhibition of DNA synthesis in WM cell lines with an IC50 of 5-20µM in all cell lines tested. Similar effects were demonstrated in 3 primary CD19+ WM cells obtained from patients' bone marrow. Cell cycle analysis demonstrated G1 arrest at 24hrs. The effects of perifosine were significant even in the presence of BM stromal cells that induce resistance. Perifosine did not induce cytotoxicity in healthy donor peripheral blood mononuclear cells indicating no toxicity on normal cells. In addition, low doses of perifosine (5µM) induced a decrease in IgM secretion in WM cells at 12 hrs of incubation. Conclusion. Perifosine possessed significant antitumor activity in WM in vitro. Future studies are ongoing. These results provide the framework to test perifosine as a new therapeutic agent in patients with WM. Supported in part by the Leukemia and Lymphoma Society, the Lymphoma Research Foundation and an American Society of Hematology Scholar Award.

0615

ANALYSIS OF DELTA AND T-CELL RECEPTOR GENES IN MYCOSIS FUNGOIDES AND SZARY SYNDROME

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Background. Demonstration of a dominant T-cell clone in Mycosis Fungoides (MF) and Sézary Syndrome (SS) is usually made with β and γ probes and rarely with Δ probes. Aims. We studied T-cell clonality for TCR Δ and γ chain gene in the peripheral blood (PB), bone marrow (BM) samples and cutaneous lesions of 14 patients with early-stage MF and in 2 patients with advanced-stage MF and SS. Method Four and five specimens were analysed: 11 skin biopsies and 14 PB samples from patients with early-stage MF, 4 skin biopsies, 28 PB cells and 7 BM samples from patients with advanced-stage MF and SS. PCR for TCR Δ gene rearrangement analysis was performed according to Hettinger et al. (1998); follow- ing amplification of γ chain, PCR products were visualised by high resolution electrophoresis on automated DNA sequencer ABI PRISM 310. PCR amplification for TCR γ gene was performed as previously reported (Ashton-Key et al., 1997), duplicated amplification products were visualised by 10% polyacrylamide gel electrophoresis (PAGE). Results. In patients with early-stage MF, monoclonality for TCR γ gene was detected in skin in 91% of the cases, in PB in 86% and both in skin and PB in 79%. TCR Δ gene rearrangements for Vα3-Jα1 and Vα1-Jα1 were observed, respectively, in 64% and 27% of cases; in 2 patients Va3-Jα1 monoclonal pattern was also associated with a Va2-Jα1 mono/oligoclonal expression. Dominant clonal TCR γ gene rearrangements were detected in skin in 91% of the cases, in PB in 50% and both in skin and PB in 22%. In 10 patients with advanced-stage MF and Sézary syndrome, TCR Δ gene analysis detected T-cell clones in the PB in 90% of cases; two molecular patterns were observed: Va3-Jα1 (80%) and Va1-Jα1 (20%). Identical molecular patterns were detected on 4 patients having skin biopsies simultaneously obtained with PB samples. Moreover we identified some patients with stable clonal pattern and some patients with multiple sequential PB and skin samples exhibiting different TCR Δ gene rearrangements (2 clinical responses and 3 progressive diseases during the follow-up period) and others with both persistent and variable clonal pattern (1 clinical response and 2 progressive diseases). Identical T-Lymphocyte clones were also detected in 7 cases in BM samples and PB specimens, with confirmation with conventional light microscopic examination of cutaneous lymph node biopsy sample. We concluded that TCR Δ gene rearrangements in advanced-stage MF and SS well correlated with TCR Δ results. Summary/Conclusion. 1) Both PCR techniques for TCR Δ and TCR γ gene showed reproducible results for detecting identical circulating...
ing and cutaneous T-cell clones in MF/SS 2) In comparison with PAGE, the TCR γ rearrangement using a PCR-RFLP probe allows a more precise assessment of the molecular pattern at diagnosis and during follow-up; all TCR Δ rearrangements were VA3-1A1 and VA1-1A1, often integrated in their mono-oligoclonal expression. Our finding of a restricted pattern of rearrangements support the suggestion of a specific antigen in MF/SS; moreover it is intriguing that some Authors consider the clonotypic TCR as a source of tumor-specific antigens and a possible target for recognition by CD8+ CTLs.

**0616 IMMUNOGLOBULIN V GENES IN WALDENSTROM'S MACROGLOBULINEMIA REVEAL ECTOPTIC SOMATIC MUTATIONS BUT NO GLYCOSYLATION MOTIFS**

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Background and Aims. In Waldenström’s macroglobulinemia (WM), current evidence from Ig VH gene analysis indicates heterogeneous disease origins. Most cases appear to derive from post-follicular B-cells with somatically mutated (MUT) VH genes, but a few WM display a few WM display a few WM display a few WM display a few WM display a few WM display a few WM display a few WM display a few WM display a few WM display a few WM display a few WM display a few WM display a few WM display a few WM display a few WM display a few WM display a few WM display a few WM display a few WM display a few WM display a few WM display a few WM display a few WM display a few WM display a few WM display a few WM display a few WM display a few WM display a few WM display a few WM display a few WM display a few WM display a few WM display a few WM display a few WM display a few WM display a few WM display a few WM display a few WM display a few WM display a few WM display a few WM display a few WM display a few WM display a few WM display a few WM display a few WM display a few WM display a few WM display a few WM display a few WM display a few WM 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tics in a range of conditions. The CD20 molecule expressed on B cells is the best validated therapeutic target for B-malignancies. Using human Ig transgenic mice, we have generated a panel of fully human CD20 mAb (HuMax) directed against the human CD20 molecule. Characterization of these antibodies revealed that two types of CD20 specific antibodies exist: type I CD20 mAb, exhibiting similar characteristics to the chimeric mAb rituximab, and type II CD20 mAb, being functionally comparable to the murine anti-CD20 mAb Tositumomab (B1). The biological property of one of the type I mAb, HuMax-CD20, has been evaluated performing in vitro and in vivo experiments. Methods and Results. In vitro experiments showed that HuMax-CD20 has an unusually slow off-rate, and induced rapid translocation of CD20 into lipid rafts. Analysis of its CDC potential showed that HuMax-CD20 recruited C1q to the surface of CD20-positive cells and mediated tumor cell lysis via activation of the classical pathway of complement. Importantly, HuMax-CD20 was exceptionally active in CDC, in the presence of human plasma or whole blood, being able to lyse a range of rituximab-resistant targets cells, such as CD20-low expressing CLL. This CDC potency appeared to be related to the unusually slow off-rate of these human antibodies. Our current data on epitope mapping indicated that the CDC potency might be influenced by the region of the CD20 recognized by HuMax-CD20. Binding by rituximab and mouse CD20 mAb, had an absolute requirement for alanine, and proline at positions 170, and 172, respectively, within the large extracellular loop of CD20. Epitope mapping studies, using both mutagenesis studies and overlapping 15-mer peptides of the extracellular loops of CD20, revealed a novel binding site required for binding of HuMax-CD20. The HuMax-CD20 binding epitope is located amino terminally of the binding site for rituximab and is also located in the extracellular loop of CD20. Thus, while off-rate may influence biological activity of mAb, the most critical factor determining CDC potency by CD20 mAb, appears to be the region within the target molecule they recognize. In vivo experiments showed that HuMax-CD20 increased survival in a SCID xenograft model. 1v. infusion of HuMax-CD20 in cynomolgus monkeys lead to a profound, long lasting B cell depletion, which recovered after the last dose. HuMax-CD20 has been selected for further clinical development. HuMax-CD20 is currently in phase I/II trials for follicular lymphoma, and B-CLL, and in a phase II trial in Rheumatoid Arthritis. Conclusion. These results indicate that HuMax-CD20 holds considerable promise for improved clinical activity and may represent an attractive candidate to treat patients with B-cell malignancies and autoimmune disease.

0620
THE INFLUENCE OF INTERLEUKIN-10 PROMOTER GENE POLYMORPHISM ON THE OCCURRENCE OF NON HODGKIN LYMPHOMA IN SUBJECTS INFECTED WITH HEPATITIS C VIRUS
A. De Renzo,1 M. Capasso,2 P. Ferna,2 E. Persico,2 C. Marzocchella,1 A. Iolascon,1 M. Persico1
1University of Naples, NAPLES, Italy; 2Second University, NAPLES, Italy

Background. HCV along with chronic liver disease is also considered a causative agent of other clinical pathological conditions which testify the possible direct pathogenic role of the virus in several different cell types including hepatocytes and leukocytes. Prevalence of HCV is significantly higher also in patients suffering with NHL and, all around the world, it was confirmed except for patients studied in North Europe and some areas of North America. In Italy, different groups showed prevalence ranging from 15 to 30%. Aims. The goal of this paper is to establish if a polymorphic gene encoding for cytokine could be a predisposing factor for this condition. Methods. To do this, we analyzed the distribution of the polymorphism of IL-10-1082 G/A in 63 patients, not infected with HCV, with Non Hodgkin Lymphoma (NHL/HCV) and in 50 patients, infected with HCV, with chronic active hepatitis, with Non Hodgkin Lymphoma, (NHL/HCV+). Results. In this study, for the first time we show that irregardless of age, sex, virus genotype and/or severity of chronic liver disease a significant prevalence of IL-10-1082 GG genotype seems to influence the occurrence of NHL in HCV infected patients. In fact the distribution of the IL-10-1082 G/A polymorphism was different between NHL/HCV+ and NHL/HCV- patients (p=0.028). The frequency of the IL-10-1082 G allele (p=0.019) and the frequency of the IL-10-1082 GG genotype against overall genotypes (IL-10-1082 GA/AA) were statistically higher in NHL/HCV+ patients as compared with NHL/HCV- patients (p=0.014).

0621
ALEMTUZUMAB FOR THE TREATMENT OF SEZARY SYNDROME: CLINICAL AND IMMUNOLOGIC FINDINGS IN 10 PATIENTS
M. Bernengo,1 P. Quaglino,1 A. Comessatti,1 M. Ortonelli,2 M. Novelli,2 T. Fierro,2 F.M. Palombi1
1University of Turin, TORINO, Italy; 2Sant’Eugenio Hospital, ROMA, Italy

Background. Sézary syndrome (SS) is a cutaneous T-cell lymphoma, for which chemotherapy resistance is a major obstacle for effective therapy. Alemtuzumab (Campath®, MabCampath®) has been shown to be effective for the treatment of SS, but often with severe hematologic toxicity and an increased risk of infection. We determine if an altered, patient-specific regimen of subcutaneous (SC) alemtuzumab can induce hematologic, immunologic, and clinical responses similar to that achieved in response to the standard regimen in patients with SS, while reducing treatment-related toxicity. Methods. Alemtuzumab was administered as follows: 5 patients received 3 mg on Day 1, 10 mg on Day 5, and 15 mg on alternating days thereafter; 6 patients received 3 mg on Day 1 and 10 mg on alternating days thereafter; 1 patient received 1 mg on Day 1, 3 mg on Days 3 and 5, and 6 mg on Day 7. Alemtuzumab was administered until the number of circulating Sézary cells was <1,000/mm³. Peripheral blood lymphocytes were monitored for Sézary cells by 3- or 4-color flow cytometry each day before alemtuzumab treatment until there were <1,000 cells/mm³. Clinical and immunologic responses were measured every 2 weeks for the first 2 months, and monthly thereafter. Results. Ten patients (median age 72 years [range, 48-82]) with SS were enrolled in this study; 5 patients with relapsed/refractory disease after ≥1 prior therapies, and 2 patients with newly diagnosed disease with a high peripheral blood cell count. Treatment with alemtuzumab resulted in a median decrease of 96% (range, 78%-99%) in Sézary cells, with 9/10 patients having <1,000/mm³ in 4-13 days after the start of treatment. Clinical responses were achieved in 9/10 patients (1 CR, 8 PR), and 1 patient had SD after 4 weeks of therapy. Median survival was 13 months (range, 5-32 months); 2 patients died due to progressive disease and 1 from infectious complications. After a median follow-up of 14 months (range, 5-32 months), 2 patients relapsed after 4 and 7 months, respectively, while 7 patients remain in remission. Hematologic toxicity was mild; 1 patient developed thrombocytopenia grade 2/3 and anaemia grade 1. Cytomegalovirus was detected in 3 patients during follow-up, which resolved after intravenous (IV) ganciclovir treatment. Three patients developed sepsis that resolved with IV antibiotics in 2/3 patients, while the other patient died from infectious complications. Infectious complications occurred in all 4 patients who were treated with alemtuzumab 15 mg, but did not occur in any patients treated with the 10-mg dose (p=0.0048). A significant decrease in the absolute values of the normal CD8+CD+ cells was observed immediately after the first cycle (p=0.002); however, the percentage of NK and CD8+CD+ cells increased significantly after the first cycle (p=0.006 and p=0.002, respectively). Summary/Conclusion. Subcutaneous alemtuzumab, administered at a schedule that is based on the number of circulating Sézary cells, was well tolerated and resulted in a high response rate with durable remissions in patients with Sézary syndrome.

0622
LENALIDOMIDE INHIBITS PROLIFERATION OF THE CHROMOSOME 5 MUTANT HEMATOPOIETIC TUMOR CELL LINE NAMALWA CSN.70 AND INTERFERES WITH RECEPTOR SIGNALING THROUGH THE SRC/GRB2/GAB1/AKT PATHWAY
A. Gandhi, J. Kang, S. Naziruddin, A. Parton, P. Schafer, D. Stirling
Celgene Corporation, SUMMIT, USA

Background. Lenalidomide (Revlimid®) has recently been approved for the treatment of a subset of myelodysplastic syndromes (MDS) and is currently being evaluated as treatment for a broad range of other hematologic and oncology conditions, including multiple myeloma, chronic lymphocytic leukemia and solid tumor cancers. Lenalidomide efficacy has been reported in clinical trials of MDS patients with a 5q- cytogenetic abnormality, with or without other cytogenetic abnormalities. Aims. The present study examines the molecular mechanism of action of lenalidomide in the chromosome 5 deleted Burkitt’s Lymphoma tumor cell line, Namalwa CSN.70. Methods. Karyotype Analysis. Lenalidomide was evaluated as a single agent using 48-color multiphoton microscopy to identify specific chromosomal events. Cell proliferation assay. Cells were incubated in 96-well cell culture plates with compounds for 72 hours and assayed by 3H-thymidine incorporation. IC50s were calculated by nonlinear regression analyses with GraphPad Prism. Immunoblot and immunoprecipitation. Cells were treated then stimulated with Epo (R&D Systems). Lysates were immuno-
Epstein-Barr virus (EBV, human herpesvirus type 4) is ubiquitously distributed in all human populations, reaching infection rates of more than 90%. EBV is known to infect B-lymphocytes and mucosal epithelial cells and to establish latent or productive infections. The virus is the causative agent of infectious mononucleosis and closely associated with various lymphoid and epithelial malignancies, such as the endemic form of Burkitt lymphoma. EBV has also been associated with the development of various lymphoid malignancies and epithelial tumors. In addition, EBV has been shown to be a causal agent of several other diseases, including gastric cancer, nasopharyngeal carcinoma, and possibly some cases of cervical cancer.

**Results.** In vitro, B-lymphocytes are transduced with the EBV genome, and infected cells can be detected by flow cytometry using specific antibodies against EBV antigens. The EBV genome is integrated into the host cell genome, and the integration site can be determined by Molecular Analysis (Molecular Designs).

**Conclusions.** These studies provide evidence that the mechanism of action of lenalidomide in this transgenic model is mediated through the inhibition of the Akt signaling pathway. The combination of lenalidomide and immunotherapies is an attractive approach for the treatment of various hematological malignancies.

**Table 1. Discrepant cases when FCM, cytology, and/or histology techniques were compared (n=29).**

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<th>Histopathology</th>
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**Background.** At present, diagnosis of chronic lymphoproliferative disorders (CLPD) on lymphoid tissue samples is performed by a combination of different techniques; histopathological and immunohistochemical analyses are considered the reference approaches. To date, large studies evaluating in a prospective way the reliability of flow cytometry (FCM) for the screening of CLPD by comparison with both cytologic and histologic diagnoses in the same fine-needle aspiration (FNA) samples remain scarce. Aim. To establish the utility of FCM for the screening of CLPD from lymphoid tissue samples obtained by FNA.

Materials and Methods. A total of 263 correlative samples obtained by FNA were stained with CD8-BTC/PE/CD4-CD19-PC5/CD3-APC (screening tube). Reference microbeads (Perfect Count) were added, to calculate the absolute numbers of cells/sample. Data acquisition was performed on a FACSCalibur flow cytometer. Data obtained by FCM were compared with those obtained by cytological and histological techniques. For the discrepant cases, the final diagnosis was based on either molecular/genetic or histological analyses. In the statistical comparisons, p values <0.05 were considered to be statistically significant. Results. In 228 samples (87%), FMC was concordant with the diagnosis made by cytology and histology.
In 69 out of the 228 samples (30%), the final diagnosis was compatible with B-non-Hodgkin Lymphoma (B-NHL) (53 cases sIgkappa+, 15 sIgLambda+ and one case sIgGamma), 113 cases (49%) were considered as reactive processes (RP; n=97) or Hodgkin disease (HD; n=16, confirmed by histology) and in 27 (12%) infiltration by non-hematopoietic cells was detected (solid tumor -ST- by histology). In the remaining 19 cases (8%), the diagnoses corresponded to: plasmacytoma (n=7), T-cell CLPD (n=6), T-acute lymphoblastic leukemia (T-ALL; n=5) and acute myeloid leukemia (AML; n=1). Discrepant samples (n=25) corresponding to 29 cases are shown in Table 1a and 1b. In 14 out of these 29 cases (48%) the final diagnosis was discordant with that provided by FCM, while in 5 (10%) cytology gave the correct diagnosis, in the remaining 12 cases (31%), the diagnosis was not conclusive, mainly due to low cellularity or peripheral blood contamination. The sensitivity and specificity of FCM in diagnosing the different CLPD ranged between 94-100% and 88-100%, respectively. Overall, in B-NHL the percent of clonal B-cells was significantly higher than in the other groups (59±25% vs 8%), all these cases showed an imbalance in the k:lambda ratio. Also, a significantly higher percent of T-cells was found in T-NHL, as compared to the other groups -except AML- (59±24% vs 36±25%). In ST, a variable infiltration by non-hematopoietic cells was found (58±34%); the diagnosis of plasmacytoma, dendritic cell (DC) neoplasia, T-ALL and AML was based on the identification of increased numbers of plasma cells (55±38%), DC precursors (9±25%), large blast cells (8±5%) and myeloid blast cells (96%), respectively. Of note, the diagnosis was established from relatively low numbers of cells (1.9±3.6 x10^6). Conclusion. Screening by FCM of lymphoid tissue samples obtained by FNA is a precise, fast and cheap tool to be used for the diagnosis of CLPD, requiring a relatively low numbers of cells, although it is not useful for the diagnosis of HD.


Background. Anaplastic Large Cell Lymphoma (ALCL), a rare Non-Hodgkin’s Lymphoma (NHL), with less than 5% incidence, has a predilection for younger patients, disseminated disease at presentation, and extranodal determination. A standardized treatment is not yet available. Aims. The aims of our study are to analyze the clinical and pathological correlation of 45 cases of T and null ALCL. Methods. This is a multicentric retrospective clinical and pathological study of 45 cases diagnosed in our hospitals between 1997-2004; a lot of monoclonal antibodies and techniques were utilized for phenotypic evaluation by immunohistochemical stainings. Results. Of note, the diagnosis was established from relatively low numbers of cells (1.9±3.6 x10^6). Conclusion. Screening by FCM of lymphoid tissue samples obtained by FNA is a precise, fast and cheap tool to be used for the diagnosis of CLPD, requiring a relatively low numbers of cells, although it is not useful for the diagnosis of HD.

0626 EXPRESSION OF CD52 IN T-CELL AND NK/T-CELL LYMPHOMAS S.S. Chuang Chi-Mei Medical Center, YUNG-KANG CITY, Taiwan

Background. CD52 antigen, also known as CAMPATH-1, is a heavily glycosylated small peptide linked to the surface membrane. It is expressed in high density by lymphocytes, monocytes/macrophages, eosinophils, thy- mocyes and macrophages, but is absent in granulocytes, platelets, red cells, and bone marrow stem cells. CAMPATH-1H or Alemtuzumab is a genetically reshaped human IgG1 monoclonal antibody against CD52. It has shown to be effective in T-cell malignancies, particularly in T-cell prolymphocytic leukemia and cutaneous T-cell lymphoma (TCL). It is also approved for the treatment with psoriasis and Sézary Syndrome (SS). We have been heavily pretreated with conventional chemotherapy, although it is associated with significant hematologic toxicity and infectious complications. To date, there have been very limited studies focusing on the expression of CD52 in TCL. Aims. To investigate CD52 expression in TCLs. Methods. Immunohistochemical study using anti-CD52 (MC1-642, Serotec, Oxford, UK) in 47 T-cell lymphomas. Results. CD52 expression in 20% of tumor cells is considered positive. Results. CD52 is expressed in 35 (86%) cases including 10/14 (71%) angioimmunoblastic T-cell lymphomas (AITLs), 15/34 (44%) unspecified PTCLs, 4/17 (24%) NK/T-cell lymphomas, 5/18 (17%) anaplastic large cell lymphomas, 1/10 (10%) T-cell lymphoblastic lymphomas, 1/1 panleukemic TCL, 1/1 adult T-cell leukemia/lymphoma, but not in one hepatosplenic TCL. Summary/Conclusions. Our results show that one third of T-cell malignancies express CD52 with various frequency among various subtypes and suggest that AITL and unspecified PTCL are better candidates than other subtypes for CAMPATH-1H treatment. It might be advisable to perform CD52 immunostaining before starting CAMPATH-1H treatment.

0627 SPLENIC MARGINAL ZONE LYMPHOMA: ONE OR MORE ENTITIES? A HISTOLOGICAL, IMMUNOHISTOCHEMICAL AND MOLECULAR STUDY OF 42 CASES T. Papadaki1, K. Stamatopoulos2, C. Belessi3, E. Pouliou1, A. Parasi3, V. Douka1, A. Hadzimidritniou1, N. Lacoutaris1, A. Fassas1, A. Anagnostopoulou2, D. Anagnostou1
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The histogenesis of splenic marginal zone lymphoma (SMZL) is currently unknown. We conducted a detailed histological, immunohistochemical and genotypic analysis in a series of 42 SMZL cases, diagnosed on splenectomy specimens after established WHO criteria. A broad spectrum of monoclonal antibodies was used in order to exclude other small B-cell malignancies mimicking SMZL, in 12/42 cases with T-cell leukemia/lymphoma, but not in one hepatosplenic TCL. Our results show that one third of T-cell malignancies express CD52 with various frequency among various subtypes and suggest that AITL and unspecified PTCL are better candidates than other subtypes for CAMPATH-1H treatment. It might be advisable to perform CD52 immunostaining before starting CAMPATH-1H treatment.
IGHV-mutated genes were SlgD-negative; in contrast, IGHV-unmutated cases were expressed SlgD (7/12, 58%). CD27 was detected at a sim-ilar frequency in either the IGHV-mutated or IGHV-unmutated sub-groups (11/16 and 8/12 cases, respectively). Six out of 10 CD27-negative cases carried IGHV-mutated genes; all six CD27-negative/IGHV-mutat-ed cases expressed DBA.44. These results confirm the considerable his-tological, immunohistochemical and molecular heterogeneity of SMZL and indicate that the detected diversity is not restricted to the disease population during the normal SMZ. Furthermore, they indicate a role for selective antigenic pressures in the pathogenesis of at least a subset of SMZL cases.

0628

REAL-TIME QUANTITATIVE PCR AND FOUR COLOUR FLOW CYTOMETRY FOR MINIMAL RESIDUAL DISEASE ASSESSMENT OF MANTLE CELL LYMPHOMA

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Background. MCL is an aggressive disease and few patients reach long-term survival. The impact of tumour load estimation and MRD quantifi-cation in these patients is unclear. We analysed peripheral blood (PB) and bone marrow (BM) disease levels obtained by both FC-PCR and FC in 15 patients with MCL. 12 males and 3 females, median age 61 yo (range 55-85), all in stage III or IV, for whom a BM (14 pts) or PB (1 pt) sample was obtained at diagnosis. MCL was diagnosed by lymph node biopsy in all cases. Fourteen patients were treated with therapeutic protocols including anthracyclines in 11, with (N=7) or without (N=4) Rituximab, and clo-rambucil+prednisone in the remaining 4 patients. Methodology: 2-CDA was analysed from BM samples, either at time of diagnosis (18/31), after (7/31) or during (4/31); 1st line therapy or during complete remission (1/31). FC PCR quantification was performed using amplification of the BCL1-IGH (Hirt C et al., Blood 2004) in 3/15 pts and using an ASO against the IGH-CDR3 (Verhegan OJ et al., Leukemia 2000) in 8/15 pts or on both tar-gets in the remaining 4/15 pts. FC was performed using the following panel: anti CD19, CD20, CD22, CD23, CD10, FMC7, CD5, CD45, K, L B cells were considered neoplastic if they were CD19+/CD5+ and exhibited light chain restriction; CD23 and CD10 were always negative. 20000-150000 events were acquired per sample. Results. At diagnosis (N=15) FC sensitivity was 100% (10-4 and 10-5), whereas RQ-PCR sensitivity between 1x100 and 1010 demonstrated tumour infiltration in all samples analysed. Importantly, in 4 BM samples without histological infiltration, FC and RQ-PCR detected disease levels between 1.2-30.2% and 2.6-19%, respectively. Overall we obtained concordant results between RQ-PCR and FC in 11/15 (73%) cases. Discrepant results were noted in 2 cases, one who had a low level of disease detected by both FC and RQ-PCR which was only detected by RQ-PCR. The remaining 2 cases are under further investigation. Conclusion. The value of gene expression was normalised to the calibrator (healthy tissue cells). Results. hCNT1, RR2, 5-NT gene expression analysis has shown lower values in patients than in healthy tissue controls. 2 patients who achieved clinical partial remission (PR) presented 100 times lower hCNT1 levels (median 3x10-2) and 100 times lower hCNT1 levels (median 3x10-2, range 0.1-9) than patients (n=12) in complete remission or very good partial remission (median 2x10-2, range 0.1-9x10-2, p=0.03). Three patients showed drug toxiciti-ty: 2 of them (with very low hCNT1 levels) interrupted 2-CDA treatment. A complete remission and clinical CR accounted for 95% for hCNT1 expression and both approaches should be considered for future clinical studies. hCNT1 expression was not predictive of lack of clinical activity of 2-CDA. However the lower hCNT1 expression detected in patients who achieved only PR could suggest a possible relationship between reduced hCNT1 expression and a diminished clinical activity of 2-CDA. Thus it might be important to explore the possibility of standardizing a quantitative method in order to identify a threshold value which could be predictive of drug resistance.

0630

LYMPHOMA CELLS MICRODISSECTED FROM PRIMARY SPLENIC LOW GRADE NON-HODGKIN’S LYMPHOMA BUT NOT NORMAL SPLENIC CELLS CONTAIN HCVC GENE

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The role of HCV infection in the pathogenesis of various types of B cell Non-Hodgkin’s lymphomas (B-NHL) has been suggested from epidemiological studies, however molecular mechanisms accounting the neoplastic transformation have been to be determined. To investigate the relationship between HCV infection and B-NHL we studied spleen paraffin-embedded sections of 12 patients with primary splenic B-NHL. B-NHL patients were affected from a high grade B-NHL, 4/12 from a low grade B-NHL. Normal and neoplastic cells were individually microdis-sected as pure cells populations from tissue sections by using a Laser-assisted microdissection apparatus. The presence of HCVC genomes was investigated by RT-PCR in RNA extracted from these two cell popula-tions of each patient. While the HCV genome was found both in neo-plastic and in normal cells in all the eight patients with high grade disease, the four low grade B-NHL samples showed the presence of the HCV genome only in the lymphoma cells and not in the normal cells. These results suggested a direct role of the virus in the low grade lym-phoma transformation. In addition, to investigate the molecular path-ways involved in the neoplastic transformation, we evaluated, by quan-titative real time PCR, BCL2 expression in the cell populations microdis-sected from the patients. Interestingly, lymphoma cells isolated from low grade NHL patients showed very low expression of the BCL2 gene thus suggesting that molecular mechanisms of neoplastic transformation in these patients is not BCL2-mediated as in the other low grade NHLs. In conclusion, our results indicate that at least in low grade NHLs, the HCV might directly involved in the neoplastic transformation, and that the molecular mechanisms may be different respect the other types of low grade NHLs.
Acute myeloid leukemia III

0631
APPLICATION OF EXTENDED COX PROPORTIONAL HAZARD MODELS ON THE DATA SETS OF THE ORIGINAL DATA BASED META-ANALYSIS ON PATIENTS 18 TO 60 YEARS OF AGE WITH COMBINATION-FACTOR ACUTE MYELOID LEUKEMIA OF THE GERMAN AML-INTERGROUP

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Background. There is increasing interest to apply survival analysis to data sets with multiple events per subject including on the one hand multiple events of the same type and on the other hand events of different types. In our meta-analysis on CBF-AML (Schlenk et al. JCO 2004, 22:3741) we used preponderantly time to first event approaches like relapse free survival. However, such approaches neglect the multiplicity of events and especially the influence of treatment after relapse if relapse is taken into account. Aims. We performed a re-analyses of the datasets of our meta-analysis using extended Cox proportional hazard models to assess the impact of different treatment strategies during first remission (CR) and after relapse in a unique model. Methods. We used three approaches of extended Cox models for the re-analysis: i) the Andersen-Gill (AG) model assuming independence of events in the different time periods first CR and after relapse, ii) the Prentice-Williams-Peterson (PWP) model assuming that a patient can only be at risk for the second time period after relapse until he underwent an event in the first time period first CR, iii) the Wei-Lin-Weissfeld (WLW) model allowing a separate underlying hazard for each event. The different models were compared by explained variation using the Brier-Score. The dataset were restricted to full-set records for patients achieving a first CR (inv(16) n=158, t(8;21) n=149). The variables in the models were the different treatment strategies (allogenic transplantation from matched-related donor (ALLO-SCT), allogeneic transplantation from matched-unrelated donor (MUD-SCT), intensive high-dose cytarabine based chemotherapy (CHEMO), autologous transplantation (AUTO-SCT)), trisomy 22 (inv(16)), loss of X or Y (t(8;21)), disease state (CR versus no-CR), dichotomized (>25.4) WBC count (t(8;21)) and dichotomized (>28) platelet count (t(8;21)). Results. The explained variation for the different models in the inv(16)-data set were 0.105 for the AG-model, 0.110 for the PWP-model and 0.058 for the WLW model and in the t(8;21)-data set 0.261 for the AG-model, 0.226 for the PWP-model and 0.161 for the WLW model. The models with highest values in explained variation was ALLO-SCT 0.39 (95%-CI 0.20-0.77), MUD-SCT 0.25 (95%-CI 0.07-0.87), AUTO-SCT 0.63 (95%-CI 0.37-1.06), trisomy 22 0.50 (95%-CI 0.26-0.95) and disease state 0.43 (95%-CI 0.14-1.30) for the inv(16)-data set and for the AG-model were ALLO-SCT 0.39 (95%-CI 0.20-0.77), MUD-SCT 0.25 (95%-CI 0.07-0.87), AUTO-SCT 0.58 (95%-CI 0.24-1.4), MUD-SCT 3.4 (95%-CI 1.2-9.5), loss of Y 1.65 (95%-CI 1.02-2.7), WBC<25.4/mL 0.4 (95%-CI 0.23-0.7), platelets<28.2 (95%-CI 1.33-3.8) and disease stage 0.2 (95%-CI 0.1-0.43) for the t(8;21)-data set. Conclusion. From the statistical point of view extended Cox proportional hazard models can be well applied to datasets of AML patients. From the clinical point of view the beneficial effects of ALLO-SCT in inv(16)-AML raises the question of treatment strategies in first CR and in t(8;21)-AML the very strong impact of the dichotomized WBC and platelet count at diagnosis argues for risk stratification.

0632
ALL-TRANS RETINOIC ACID AND GENTUTUMAB OZOGAMICIN AS ADJUNCT TO SALVAGE THERAPY IN PRIMARY REFRACTORY ACUTE MYELOID LEUKEMIA: RESULTS OF CONSECUTIVE PHASE II STUDIES OF THE AMLSG

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Background. Response to first induction therapy is one of the most important prognostic factors in patients with adult myeloid leukemia (AML). Induction of CR or PR is the primary aim in these patients. Aims. To evaluate the impact of all-trans retinoic acid (ATRA) and gentuzumab ozogamicin (GO) given as adjunct to intensive salvage therapy in primary refractory younger patients on clinical outcome. Methods. Between 1995 and 2005 255 consecutive patients (median age: 48 yrs, range 16-60 yrs) treated within the AMLHD93 (n=45), AMLHD98A (n=160) and AMLSG 05-04 (n=50, still active) were evaluated. All patients had primary refractory AML after one cycle of ICE (idarubicine, cytarabine, etoposide). The different salvage therapies were as follows: AMLHD93 sequential-HAM (S-HAM) for patients <25 years of age [cytarabine 3g/m² bid. days 1,2,8,9, mitoxantrone 10 mg/m² days 3-4,10,11], HAM for patients ≥25 years of age [cytarabine 3 g/m² bid., days 1-3, mitoxantrone 12mg/m² days 2,3], AMLHD98A: 3 g/m² days 3-5, 15 mg/m² days 6-28; AMLSG 05-04: GO-HAM [HAM with ATRA and GO], A-HAM with gemtuzumab ozogamicin 3 g/m² /day 1). Results. The distribution of the different salvage therapies was HAM n=21, S-HAM n=22, A-HAM n=118, GO-HAM n=53, other n=31 no further therapy n=10. Response according to salvage therapy was as follows:

<table>
<thead>
<tr>
<th></th>
<th>GO-A-HAM</th>
<th>A-HAM</th>
<th>S-HAM</th>
<th>HAM</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR</td>
<td>26 (53%)</td>
<td>40 (34%)</td>
<td>5 (23%)</td>
<td>3 (14%)</td>
</tr>
<tr>
<td>PR</td>
<td>6 (12%)</td>
<td>33 (28%)</td>
<td>5 (23%)</td>
<td>4 (19%)</td>
</tr>
<tr>
<td>RD</td>
<td>15 (31%)</td>
<td>36 (31%)</td>
<td>12 (54%)</td>
<td>12 (57%)</td>
</tr>
<tr>
<td>death</td>
<td>2 (4%)</td>
<td>8 (7%)</td>
<td>0 (0%)</td>
<td>2 (10%)</td>
</tr>
</tbody>
</table>

No CTC-grade 3-5 liver toxicity was seen in patients receiving GO-A-HAM. Logistic regression on the achievement of CR after salvage therapy revealed that regimens containing ATRA (odds ratio 2.0, p=0.05) and GO (odds ratio 2.2 p=0.02) were associated with response. 142 of 255 patients have received stem cell transplantation. One (4%, 95%-CI 0.002-0.18) case of severe veno occlusive disease was seen in 28 so far transplanted patients who have had GO-A-HAM. Median survival was 11.3 months. Conclusions. Although retrospective in nature our study suggests that ATRA and GO as adjunct to salvage chemotherapy in primary refractory AML patients improves CR rates.

0633
GENETIC CHARACTERIZATION OF PATIENTS WITH AML-M2. THE JAK2 V617F ACTIVATING MUTATION IS FREQUENTLY FOUND IN CASES WITH NORMAL KARYOTYPE

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The characterization of genetic and molecular aberrations in AML has substantially improved our understanding of the pathogenesis of this disease. The activating V617F mutation of JAK2 has been recently described as a common event in MPD. The same mutation was also found in a small number of patients with either AML or MDS; however, there are few data about the frequency of JAK2 V617F in specific subtypes of AML. We investigated the incidence of this mutation in 10 cell lines and 331 well characterized AML patients, and its association with other factors with a prognosis meaning. V617F genotyping was performed by ARMS as previously described (Jones et al., 2005). All cases...
es tested positive were identified by sequencing. Mutations of FLT3 and KIT were also analyzed. A high resolution 50K SNP array (Affymetrix) was used to analyze 19 samples. We found the mutation V617F in 3% of overall AML (10/331); 2 M1 (2/71;2.8%), and 8 M2 (8/649;9.5%), suggesting a correlation with less differentiated leukemias. According to the WHO-classification, the mutated cases were: AML with t(9;21) (1/166;6.2%), AML with inv(16) (1/122;8.5%), AML with mult- itilinized overexpression of Wnt-1 (1/25;3.5%). AML without muta- tion (2/665;3%), and AML with mutation (5/58;8.6%). In 9 of these patients we could analyze other samples in CR or relapse, and we found that the V617F mutation is not a good marker for MRD. Although in 7 cases the mutation disappeared in CR and appeared again when the patient relapsed, in one patient we detected V617F in CR, and in the other there was no mutation in the relapse. As expected, 22.6% (11 of 49) AML and 50% of cases with normal karyotype, had FLT3 mutations. To further characterize the AML-M2 cases, we analyzed KIT (exons 8 and 17) in 64 patients. In 3 cases (4.7%), a mutation affecting codon 816 of KIT was detected: one D816H and 2 D816V, all with ε816; (5/12,25%). No exon 8 mutations were found. Interestingly, one patient had mutations in both JAK2 (V617F) and KIT (D816V), and another had both JAK2V617F and ITD- FLT3, showing that in some cases 2 mutations in TK genes could collaborate in the AML transformation. The 50K GeneChip array was used to type DNA from 17 AML samples (8 with JAK2 mutated, and 9 no muta- ted), and 2 cell lines with JAK2V617F five cases (29%), and the 2 cell lines, had regions of uniparental disomy (29%), confirming the impor- tance of this mechanism in AML. The 3 samples with V617F in homozy- gosis had LOH by UPD in 9p. The data presented in our study confirm the association of KITD816 mutations and ε816; whereas mutations of JAK2 and FLT3 were more frequent in samples with normal kary- type. AML samples with normal karyotype, the incidence of the V617F mutation was 10.8% (5/46). Two patients presented mutations in 2 different TK genes. Normal karyotype AML is a heterogeneous group with different molecular mutations, and a bet- ter subclassification of this group may be needed to work out a poten- tial prognostic impact of TK mutations.

0634

PREFERENTIAL METHYLATION OF WNT INHIBITOR FACTOR-1 IN ACUTE PROMYELOCYTIC LEUKAEMIA: AN INDEPENDENT POOR PROGNOSTIC FACTOR

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Background. The Wnt pathway is activated frequently in acute leukemia. Aim to study the role of methylation of regulatory molecules in acute leukemia. Methods. The epigenetic suppression of Wnt-1, a neg- ative regulator of Wnt, was studied in five leukemic cell lines and 100 acute leukemia samples by methylation-specific PCR. Results. At diag- nosis, Wnt-1 was methylated in 25% (5/20) of acute lymphoblastic leukemia. Interestingly, in acute myeloid leukemia (AML) Wnt-1 methylation was found only in acute promyelocytic leukemia (APL) and not in other subtypes of AML (15/52, 47% versus 0/90, p=0.001). In the study of cases with hemizygously methylated Wnt-1, treatment with the demethylating drug 5-azacytidine led to progressive increase in Wnt-1 expression. In patients with APL, Wnt-1 methylation was associ- ated with younger median age (p=0.05) and higher presentation leu- coyte count (p=0.003). The 3-year disease-free survival (DFS) of APL patients with Wnt-1 methylation was significantly inferior to patients without Wnt-1 methylation (36.0% versus 74.1%, p=0.005). In multivari- ate analysis, Wnt-1 methylation. Conclusion. Wnt-1 is preferably methylated in APL, and may be an adverse prognostic factor.

0635

IMPLICATIONS OF REAL TIME FLIP GENE EXPRESSIONS ON THE CLINICAL OUTCOME IN ADULT PATIENTS WITH AML


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Background. Little is known about the expressions of Fas associated death domain-like interleukin 1x-converting enzyme-like inhibitory pro- tein (FLIP) in normal hematopoietic in addition to leukemic cells. Many prognostic parameters have been developed to find a specific molecular explanation for unresponsiveness to induction chemotherapy (IC) in adult patients with acute myelogenous leukemia (AML). However, they should be considered on the basis of pathogenetic heterogeneity of AML. Aims. Among several proteins in association with the process of anti- apoposis on multiple levels by several regulatory mechanisms as a pri- mary cause of treatment failure in AML, we focused on the levels of expression of FLIP. An attempt was made to correlate clinical outcomes with the expression of FLIP during IC because expression differences may be potentially important for predicting of response. Methods. A total of 76 bone marrow (BM) and peripheral blood (PB) samples from 32 fresh AML patients were collected after obtaining informed consent. Separate- ly, 5 normal hematopoietic stem cell transplantation donors were used as a control group. The expressions of FLIP were analyzed after induction chemotherapy day 7, and after 21. Real-time PCR (RQ-PCR) was performed on an iCycler using iCycler software 2.1 (Bio-Rad, USA). RQ-PCR experiments were performed in duplicate, but if the FLIP-Actin values were discordant or inconsistent with RT-PCR result, the procedure was repeated. The initial response to IC together with each patient’s FLIP expression levels was examined compared to the levels of normal popu- lation. Clinical profiles and results of RQ-PCR during treatment were compared. Results. Overall, AML patients, specifically at initial diagnosis, showed relatively higher FLIP levels of expression both in the BM and in the PB. BM showed relatively higher expressions than the PB in AML. Two significant values in association with clinical outcome were the levels of expression of FLIP at initial diagnosis and on day 7 BM/PB during IC. The most reliable one was at day 7 PB (p=0.02) and patients with complete remission (CRs) showed comparable expressions with those of normal control. An unexpected finding was that the expressions after immediate recovery had no correlations with outcome. The maximum expression difference of FLIP between CRs and poor responders was 1.2 log levels by RQ-PCR. Conclusions. Although further investigations with more patients are needed to verify the exact role of FLIP in a minor cell population as shown in this study, specifically at day 7 during IC, FLIP may be an early prognostic clue for predicting of response of the treat- ment of AML patients, based on expressions of FLIP RQ-PCR.

0636

EXPRESSION OF THE BRAIN AND ACUTE LEUKEMIA CYTOMATERIAL GENE IN ACUTE MYELOID LEUKEMIA


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Clonal chromosome abnormalities play a major role in de novo AML being absolutely required to make a correct diagnosis and an accurate prognostic stratification. However, about 45% of AML patients are kary- otopically normal and are supposed to have an intermediate prognosis even though only 40% of them are long-term survivors. Therefore, in patients with new molecular markers of prognostic significance have been actively searched and recent evidence suggests that BAALC gene expression levels are one of the most relevant. In the present study we determined BAALC expression in the pre-treatment bone marrow samples of 25 adult AMLs (7 M0-M1, 3 M2, 14 M4-M5 and 1 M6); 6 females and 19 males (median age 52-75). We showed that BAALC expression is significantly lower in normal karyotype, 2 a del[7](q31q35), 2 a del(5)(q23q33) [one with +8], and 3 miscellaneous defects. The study was aimed at detecting the incidence of high BAALC expression and at correlating BAALC expres- sion levels with clinical/biological parameters and outcome. Statistical analysis were carried out by applying the method of Pfaffl HW & Dempfle L (Nucleic Acids Res 2002;30:536). BAALC relative quantification was achieved with real-time PCR using SybrGreen I as a double-stranded DNA-binding fluorescent dye. The forward and reverse primers used were those already published (Baldus et al., JCO 2006;24:790). Standard curve for real-time quantification was obtained by serial dilution of total RNA from an AML patient exhibiting an elevated BAALC expression. Quantification was achieved by applying the DDCt method. BAALC expression was normalized to ABL1 gene and calibrated on a normal control sample. A reference interval for BAALC expression quantification was fixed at 0.609 (mean expression) + 2.07 (three times the standard deviation [0.134]) after having analysed 12 normal controls. BAALC expression was low in 11 patients (median ± SD = 0.158±0.561) and high in 14 (median ± SD = 5.427±11.691) with a statistically significant difference (p<0.001). No difference between the two groups was noted in pre-treatment age, sex, white blood cell count and percentage of bone marrow blasts. High BAALC expressers were predominantly of M4-M5 cytotype. Six of the 11 chromosomally normal patients were low expressers, whereas the 3 patients harbouring a single inv(16) and all the 3 with +8 were high expressers. Nine low expressers received induction
chemotherapy: 6 achieved a complete remission (CR) and 3 did not respond. Five of the 6 CRs relapsed and were unable to achieve a new CR. Eleven high expressers received induction chemotherapy: 8 achieved CR and 3 did not respond. Four of the 8 CRs relapsed but succeeded in achieving a second CR. In Conclusion, 56% of our AML patients presented a BAAALC expression significantly higher than that of the remaining 11 patients; a high BAAALC expression correlated with +8 and inv(16) risk groups (p13q22); high BAAALC expressers showed a CR duration and an overall survival longer than those of low expressers perhaps because of the higher occurrence of +8 and inv(16) in the first patient group.

**THE OUTCOME OF POSTREMISSION TREATMENT FOR AML WITH FAVORABLE CYTOGENETICS IN FIRST REMISSION**

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**Background.** The beneficial impact of high-dose cytarabine (HDAC)-based consolidation chemotherapy in acute myeloid leukemia (AML) is much greater in patients with favorable cytogenetics (t(8;21), inv(16) and t(16;16)) than in those with normal karyotypes. However, in MRC AML-10 study, patients with favorable cytogenetics who received autologous stem cell transplantation (SCT) had a markedly lower relapse rate than those who did not receive autologous SCT, although a high procedural mortality rate in adults resulted in being ultimately no difference in the overall survival (OS). Allogeneic SCT have not been recommended as standard therapy for AML with favorable cytogenetics due to relatively high high-risk related mortality (TRM). However, progress in SCT and supportive care over the past decades have led to gradual improvement in the TRM after allogeneic SCT. **Aims.** We try to compare the outcome of allogeneic SCT with HDAC during the first remission of AML with favorable cytogenetics. **Methods.** 50 AML patients with favorable cytogenetics (excluded AML, M3) who entered complete remission (CR) between March 1997 and July 2005 at three centers were reviewed retrospectively. Among 50 patients, 13 patients who relapsed or died during consolidation chemotherapy, received less than three cycles of consolidation chemotherapy or underwent autologous SCT in first remission were excluded. Overall, 37 AML patients over the 18 years with favorable cytogenetics who underwent allogeneic SCT or received three/four cycles of HDAC consolidation chemotherapy in first CR could be analyzed. **Results.** The median follow up duration was 48 months. The 5-year probability of disease free survival (DFS) and OS were 50.3% and 51.6%, respectively. The estimated 5-year probability of DFS (73.2% vs 21.0%)(p=0.005) and OS (71.9% vs 28.9%)(p=0.03) were significantly better in the patients who underwent allogeneic SCT than in those who received HDAC. The cumulative incidence of TRM and relapse rate were 9.5% and 18.6%, respectively. In the subset analysis, OS was better in the allogeneic SCT group than in the HDAC group in the setting of age < 35 years (5-year estimated OS: 100% vs 35.8%)(p=0.0054), but not different in age ≥ 35 years (p=0.54). The OS was statistically superior for allogeneic SCT group versus HDAC group in the setting of chromosomal abnormalities ≥ 2 (5-year estimated OS: 72.9% vs 41.7%)(p=0.007), but not in chromosomal abnormalities < 2 (p=0.38). In conclusion, in AML patients with favorable cytogenetics (especially younger age) who have a matched related donor, allogeneic SCT can be an option. Especially those who have more than 2 chromosomal abnormalities should undergo allogeneic SCT with matched related donor or unrelated donor. It is needed that AML patients with favorable cytogenetics who have a sibling matched donor are assigned to allogeneic SCT and remaining to HDAC or autologous SCT are randomly assigned.

**0638**

ARE FLT3 ITD AND D835 MUTATIONS SUFFICIENT INDICATORS FOR ALLOGENEIC TRANSPLANTATION IN ACUTE MYELOID LEUKEMIA? AN ANALYSIS OF PATIENTS FROM THE CZECH ACUTE LEUKEMIA CLINICAL REGISTER (ALERT)

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**Background.** FMS-like tyrosin kinase 3 (FLT3) is preferentially expressed on hematopoietic progenitor cells and mediates hematopoiesis, differentiation and proliferation. Two types of activating FLT3 mutations have been described in acute myeloid leukemia (AML): internal tandem duplication (ITD) of the FLT3 gene and point mutation within the activation loop of the tyrosin kinase domain, which mostly affects aspartate 835 (D835). Many studies have shown that presence of FLT3 ITD correlates with poor outcome of AML patients. The prognostic relevance of D835 mutation is less clear, although most likely it also has a negative prognostic effect on the patients with AML. So far it is not clear how to treat the patients with FLT3 ITD and D835 mutations compare to patients without these mutations and whether these patients benefit from allogeneic transplantation. **Aims.** Correlation between FLT3 mutations and allogeneic stem cell transplantation. **Methods.** To assess the prognostic relevance of activating mutations of FLT3 gene on outcome of allogeneic transplantations in AML patients, we performed an analysis of all patients with FLT3 mutations registered in the Czech Acute Leukemia Clinical Register (ALERT) from 2003 till the end of 2005. ALERT registers all adult patients diagnosed in 6 main haematology centres in the Czech Republic. **Results.** Within the mentioned period 170 patients with AML of median age 59 years (in total) were investigated for FLT3 mutation, within them 37 cases (22%; 19 men and 18 women) with FLT3 mutations (33 FLT3 ITD and 4 FLT3 D835) were found. 33 patients were suitable for analysis. 13 of these patients had allogeneic transplantation, 20 patients with mutations of FLT3 were treated with chemotherapy without transplantation. Results of the treatment of these patients were compared with the results of the group of patients without FLT3 mutation, which was according to other characteristics identical with the group of patients with FLT3 mutations (n=125).

**Conclusion.** Median overall survival(OS) of patients with mutations of FLT3 who had allogeneic transplantation was 42.5 weeks, median survival of patients with mutations of FLT3 treated only with chemotherapy was 29.6 weeks (p=0.4). Median disease free survival (DFS) of the same patients was 32.1 weeks in transplanted patients and 24.3 weeks in patients treated only with chemotherapy (p=0.6). OS of patients with mutations of FLT3 trended to patients without mutation FLT3 was not significantly different. A significant difference was found in DFS only. Patients with FLT3 mutations had DFS shorter than patients without FLT3 mutations (28.2 weeks compared to 50.2 weeks; p=0.05). Conclusions. Our results suggest that at present there is no strong evidence that FLT3 status alone influence the decision to proceed to allogeneic transplantation in AML patients. Decision to proceed to allogeneic transplantation should not be based on the FLT3 status only, but it should also consider other prognostic factors. Although the mutations FLT3 mean higher risk of relapses, according to our analysis they do not significantly influence the OS of AML patients.

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**0639**

OPTIMISATION OF A 48 HOUR IN VITRO CHEMOSENSITIVITY ASSAY FOR CD34+CD38-CD123+ LEUKEMIC STEM AND PROGENITOR CELLS

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**Background.** The majority of AML patients respond to remission-induction chemotherapy, but the relapse rate is high. Relapse is underpinned by outgrowth of leukemic stem and progenitor cells (LSPC). There is a need to develop chemosensitivity assays for LSPC. **Aim.** We aimed to establish a methodology to distinguish normal from leukemic SPC, to optimise maintenance of the LSPC phenotype in 48 hour culture and to quantify viable LSPC following culture with and without drugs. **Methods and Results.** The CD34+CD38-CD123+high phenotype was used to

![Figure 1. DFS, OS by postremission therapy.](image-url)
we aimed to compare the expression of these apoptosis-r
apy and overall survival (OS) of adult patients with AML. Additionally,
bone marrow or peripheral blood was examined in 50 AML patients (36
Intracellular expression of p73 protein in leukemic blasts isolated from
achievement after induction regimen. However
analysed proteins showed predictive impact on pr obability of CR
LSPC were still viable in the presence of fibronectin and cytokines.
absence of fibronectin and cytokines, whereas 50% of ara-C-treated
reduced the LSPC number to 15% of untreated control values in the
niche conditions on the sensitivity of LSPC to cytosine arabinoside (ara-
siderable variation between samples. We examined the effect of these
culture for 48 hours, (median 0% change), although there was con-
We found that serum-free culture medium, fibronectin-coated wells,
and CD38APC in order to quantify LSPC as a percentage of viable cells.
Protein expression was expressed by both percentage of positive cells
All measurements were performed using multi-color flow cytometry.
Expression was expressed by both percentage of positive cells and
mean fluorescence intensity. Results. Thirteen (36%) patients
achieved complete response (CR) after induction chemotherapy, 20
(56%) patients did not respond and 3 (8%) patients died in the early
post-induction period. The median time of the follow up reached 5
months (range 0.1-27). Comparing to normal CD34+ cells, AML blasts
had higher expression of p73 and Bax proteins as well as cleaved cas-
proteins. Moreover, the two flow cytometric assays in parallel for analy-
LSPC survival: in the first assay, viable bulk cells are enumerated
angiopoietin 1. We used two flow cytometric assays in parallel for analy-
forms, has recently been described in patients with AML.

Background. Acute myeloid leukemia with multilineage dysplasia
(AML-MD), recognized in the WHO classification as a major AML cate-
gen was recognized mainly according to cytogenetic risk catego-
ery 1, (n=10), including 9 (90%) AML cases with intermediate-risk
targets, the survival analysis of patients receiving intensive chemo-
therapy was performed with oligonucleotide HGU133 Plus 2.0
arrays (Affymetrix). Expression measures were summarized using RMA
methodology from the Affy package of the Bioconductor project. Unsu-
ervised two-dimensional cluster analysis of high variability genes was
done with dChip v1.3. In addition, a supervised analysis to identify
genes showing significant differential expression according to cytogenic
category was based on Limma package from Bioconductor which
employs Bayesian statistics adjusted for multiple testing. Results. The
unsupervised hierarchical cluster analysis identified two main groups of
cases, which differentiaed mainly according to cytogenetic risk catego-
ery 1 (90%), and 9 (9%) AML cases with intermediate-risk
cytogenetics, and group 2 (n=9), with predominance of high-risk cyto-
genetics (78%, p=0.0045). Genes found overexpressed in group 1 includ-
ed FLT-3 and several homeobox (HOXA3 HOXA5, HOXA7, HOXA9,
HOXA10, HOXB2, HOXB3, HOXB5, HOXB6, HOXB7,
HOXB8 and HOXB9) genes. On the contrary, relevant genes such as
MLL, MLL3, CEBPD and EVI1 were overexpressed in group 2. The
supervised analysis allowed the identification of a cluster of 92 genes dif-
differently expressed according to cytogenetic category. Thus, genes
found overexpressed in AML-MD with intermediate risk cytogenetics
included ribosomal constituents and genes involved in translation
(RPS20, LOC200916, LOC400055, EIF3S3), while diverse membrane-
receptor genes, including genes involved in the immune response
(FCGR3A, FCGR3B, IL1R2, PLXNC1, FCAR, CLEC4D, CLEC4E,
TNFRSF10C, CS1R1), were overexpressed in AML-MD associated with
high-risk cytogenetics. The survival analysis of patients receiving intensive
chemotherapy identified only cytogenetics, and not gene expression
prognostic categories, as prognostically significant (figure). Conclusions. Gene
expression profiling herein described supports the biological diversity
of AML-MD, which seems to be related to the underlying cytogenetic
abnormalities. Further studies in larger series of patients are warranted
to gain insight into the biological diversity of this disease and clinical
implications.

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Carlos III/FIS.
Background. Cardiotoxicity is a relatively frequent and potentially serious complication of hematopoiesis therapy. Anthracyclines (ANT) represent the greatest risk. Cardiotoxicity of ANT may develop during the treatment (acute cardiotoxicity) and during the follow-up (chronic and late cardiotoxicity). Various methods including biochemical markers have been recommended for monitoring of cardiotoxicity of treatment in hematopoiesis. Aims. Monitoring of cardiotoxicity of ANT in patients treated for acute leukemia with biochemical markers ‘N-terminal pro brain natriuretic peptide (NT-proBNP), cardiac troponin T (cTnT), echocardiography (ECHO) and electrocardiography (ECG). Methods. 26 adult acute leukemia patients (mean age 46.2±12.4 years, 15 males) treated with 26 cycles of ANT-based chemotherapy (CT) were studied. Cardiac evaluation was performed at the baseline (before CT), after first CT (cumulative ANT dose 135.3±22.5 mg/m2), after last CT (cumulative ANT dose 464.3±117.5 mg/m2) and circa 6 months after completion of CT (6 Mo after CT). Results. The results are summarized in the Table. Six months after CT, NT-proBNP concentrations correlated with systolic and diastolic LV dysfunction on ECHO (r=0.660; p<0.01) and (r=0.587; p<0.01). Decreased QRS voltage on ECG correlated with systolic and diastolic LV dysfunction on ECHO (r=0.660, p<0.001) and (r=0.592, p<0.001). Transforamations. Our results demonstrate acute and chronic cardiotoxicity of ANT. Clinical manifestation of cardiotoxicity in terms of heart failure developed in 2 (7.7%) patients. In asymptomatic patients, abnormal cardiac findings represent subclinical cardiotoxicity, which indicates a risk for development of heart failure (NT-proBNP elevations, diastolic LV dysfunction) and malignant ventricular arrhythmias (QTC prolongation). In regard of late ANT cardiotoxicity, further cardiology follow-up is warranted in all acute leukemia survivors.

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Background. Acute myeloid leukemia (AML) has been proposed to arise from the collaboration of various chromosomal abnormalities. These chromosomal abnormalities found in AML frequently target the transcription factor genes which can control important biological processes, including cellular proliferation, differentiation, transformation and apoptosis. Aims. We selected 15 SNPs (single nucleotide polymorphisms) of transcription factor genes to test whether they are associated with increased susceptibility to AML. Methods. This study analyzed the frequencies of 15 SNPs of transcription factor genes in 269 de novo AML patients and age- and sex-matched controls. These 15 SNPs were selected from 339 SNPs’ analysis in previous study which were confirmed to be more than 15-20% in minor allele frequency in 120 normal Korean population. Genotyping method is pyrosequencing using genomic DNA from peripheral blood or bone marrow samples. Results. ETS2 rs530 (T1874A, OR: 1.929, range; 1.391~2.663), rs711 (G16+155A, OR: 2.208, 1.596~3.504) and ELFI rs7799 (A173G, OR: 1.949, 1.825~2.867) were found to be significantly higher frequencies of mutant genotype and allele in AML patients than in control. On the other hand, ELFI rs1056820 (A107T), ZNF42 rs4756 (A351G) and FLI1 gchd03-024 (C1-1014A) showed higher frequency of mutant genotype in AML than in control but there was no significant difference in mutant allele frequencies. Conclusions. This study showed the association between specific ETS family genes such as ETS2 and ELFI transcription factors and AML prevalence. Therefore, it suggests these specific ETS gene abnormalities as a susceptibility gene in AML, and proposes a number of molecular strategies for targeting these genetic abnormalities for therapeutic intervention.

CHARACTERIZATION OF AML-M0: A SEARCH FOR TUMOR SUPPRESSOR GENES AND ONCOCGENES

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Acute Myeloid Leukemias (AML) form a heterogeneous group of hematologic malignancies partly characterized by specific translocations. Several oncogenes and only a few tumor suppressor genes (TSG) have been associated with AML. The search for TSG in leukemias has been to a certain extent neglected. In this study, we aim to better characterize the minimally differentiated AML subgroup (AML-M0). AML-M0 do not present specific cytogenetic abnormalities and generally have a poor prognosis. We studied cryopreserved cells from 52 AML-M0 patients. From this material we expanded T-cells to be used as control cells and sorted the leukemic blasts to obtain a pure tumor cell fraction. To find new TSG, we have used Affymetrix 10K SNP-microarrays to compare the DNA of the blasts with that of the control material. We searched for regions of loss of heterozygosity (LOH), as LOH is frequently the second hit resulting in the total loss of function of a TSG. Furthermore, we have screened the patients for mutations in known oncogenes such as FLT3, KIT, NPM1, NRAS, KRAS, PTPN11 and JAK2 and genes with dominant negative effect such as CEBPA. We found 16 patients with LOH of chromosome 21. Chromosome 21 harbors RUNX1, a well-known TSG frequently mutated in AML. We sequenced exons 3, 4 and 5 containing the Runt domain of RUNX1. 13 out of the 16 patients (81%) with LOH presented either a point mutation or deletion of RUNX1. Two other patients showed heterozygous mutations and another bi-allelic insertions. Thus in total 16 out of 52 patients (31%) showed mutation of RUNX1. We found 9 internal tandem duplications and another bi-allelic insertions. In total 16 out of 52 patients (31%) showed mutation of RUNX1. We found 9 internal tandem duplications and another bi-allelic insertions. Thus in total 16 out of 52 patients (31%) showed mutation of RUNX1. From these patients only 2 cases had a RUNX1 mutation. From the 52 patients only one showed an insertion in exon 12 of NPM1. All other oncogenes mentioned above are currently being screened. Areas in chromosomes 3, 4, 7, 8 and 17 show LOH of DNA regions of less than 2 Mb and candidate for LOH in these regions. Thus, further investigation is required to prove that the characterization of AML-M0 will provide a better understanding and eventually a more favorable treatment of this disease, and will give insight into the pathogenesis of other subgroups of AML.
Background. With modern chemotherapy, a complete remission (CR) is achieved in up to 90% of younger (<60 years) adult myeloid leukemia patients, but the majority of patients relapse due to persistence of minimal residual disease (MRD). Treatment strategies based on MRD status have not been established. Aim. The aim of the study was to evaluate the prognostic significance of multi-parameter flow cytometry (FCM) based MRD monitoring in relation to stem cell transplantation (SCT) in younger adult AML patients. Methods. Between July 1994 and June 2001, 62 younger adult patients (<60 years) were diagnosed with non-promyelocytic AML at Karolinska University Hospital Solna (in Stockholm). Morphological CR was achieved in 53 of 62 patients (85%). Follow-up MRD information was available in 45 CR patients (23 males and 22 females). The diagnostic flow cytometry panel included membrane CD2, CD3, CD4, CD5, CD7, CD10, CD11b, CD13, CD14, CD15, CD19, CD20, CD33, CD34, CD45, CD56, CD65, CD117, HLA-DR, and intracellular CD3, MPO and TdT. Phenotypic aberrancies were defined at diagnosis and used in follow-up samples in three color antibody combinations as custom-built probes. MRD levels were determined after induction treatment (1) and after the end of post-remission chemotherapy. Detectable MRD was defined as a distinct cluster of at least 15 dots. Sensitivity levels were determined as: a) 0.1% if 30 000 events were acquired, b) 0.05% if 30 000 events were acquired in cases with highly aberrant LAIP as co-expression of CD34 and CD7, CD14, CD56 or CD65 and CD34+/CD15+/HLA-DR, c) >0.01% if live-gate approach was used. ICE induction therapy was used in most patients (n=42). Autologous (auto-SCT) was performed as consolidation in first CR in 15 patients and allogeneic-SCT (allo-SCT) in 16 patients.

Aim 1. AML patients with detectable MRD after induction treatment (i,iii) or before SCT (ii,iv) subjected to allo-SCT had significantly longer RFS and OS as compared to patients who received auto-SCT or only conventional chemotherapy.

Results. Detectable MRD (1) did not predict relapse-free survival (RFS) and overall survival (OS) though there was a trend for longer RFS in MRD (2) negative patients (p=0.061). Improved RFS and OS were predicted only by SCT (p<0.001 and p= 0.001, respectively). To analyze in detail the impact of SCT on patient outcome, MRD positive patients were divided into 3 groups according to type of post-remission therapy: a) conventional chemotherapy, b) auto-SCT and c) allo-SCT. MRD (1) and/or (2) positive patients subjected to allo/auto-SCT had significantly better RFS and OS than patients who received only conventional chemotherapy (Figure 1). However, patients who underwent allo-SCT had a significantly better prognosis than patients who received auto-SCT. At time-point (2) 5-year RFS was 80%, 53% and 0% in allo-SCT, auto-SCT and no transplantation groups, respectively (p=0.003).

Conclusions. Younger adult AML patients who have detectable MRD at the end of post-remission chemotherapy have a dismal prognosis and for these patients allo-SCT, auto-SCT or innovative new treatment strategies should be strongly considered.
Acute lymphoblastic leukemia

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SPECIFIC INTENSIVE CHEMOTHERAPY PLUS RITUXIMAB FOR BURKITT'S LYMPHOMA OR LEUKEMIA IN HIV-POSITIVE AND NEGATIVE ADULT PATIENTS

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Background. A previous PETHEMA protocol (PETHEMA ALL3/97) proved that HIV-positive patients with Burkitt's lymphoma (BL) and Burkitt-like acute lymphoblastic leukemia (ALL3) had similar outcome than HIV-negative patients. Aims. To study the impact of the addition of rituximab to our previous protocol in terms of toxicity and efficacy, with special attention to HIV-positive patients. Patients and Methods. All consecutive patients diagnosed with BL/ALL3 between July 2003 and January 2006 received induction therapy including a pre-phase with cyclophosphamide (CPM) and prednisone (PDM), followed by cycle A (rituximab, ifosfamide, VCR, dexamethasone -DXM-, HD-MTX, ARA-C and VM-26), cycle B (rituximab, VCR, HD-MTX, CPA, DXM and doxorubicin) an cycle C (rituximab, DXM, VDN, HD-MTX, HD-ARAC and VP-16). Patients with BL in stages I or II received 4 cycles (A1,B1,C1, A1) whereas those with BL in stages III or IV or with ALL3 received six cycles (A1,B1,C1,A2,B2,C2) followed by two additional rituximab doses. CNS prophylaxis consisted of IT MTX+ARA-C+DXM given in each cycle for a total of 8 doses. Results. 31 adult patients (20 HIV-negative and 11 HIV-positive) were included. Both groups of patients were comparable for age, gender, ECOG score, BM and CNS involvement, bulky disease, LDH and albumin serum levels. Twenty-two patients had BL and 9 ALL3. Three out of 11 HIV positive patients begun treatment with HAART at the time of diagnosis and 8 were already under treatment. Median follow-up was 7 months (range 1-30). Main results of therapy are summarized in Table 1. No significant differences in CR, DFS or OS were observed between BL and ALL3 or between HIV-positive and negative patients. Grade 4 neutropenia and thrombocytopenia were constant and lasted a median of 7 days (range 2-31). Other frequent grade 3-4 toxicities were hepatic (8% of cycles), mucositis (18%) and infectious (18%). Episodes of grade 3-4 extrahematological toxicity were more frequent in HIV-positive patients (65% of mucositis, p=0.04; 65% of infections, p=0.04 and 62% of hepatic toxicities, p=NS).

Conclusions. Preliminary results suggest that the addition of rituximab to a specific BL/ALL3 treatment is also feasible for HIV-positive patients with similar results to HIV-negative patients in terms of efficacy although with higher toxicity.

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Since 2000, minimal residual disease (MRD) information at week 5 and 12 of therapy has been used for the treatment stratification in childhood ALL-BFM 2000 trial. In parallel, ALL IC-BFM 2002 has been designed by the International-BFM Group to test the morphological assessment of the early treatment response. Patients are stratified according to the blast proportion in peripheral blood (PB) at day 8 and in bone marrow (BM) at day 15 and 33 of therapy together with the age, initial WBC and the presence of BCR/ABL and MLL/AH fusion. Aims. One of the research questions of the ALL IC-BFM 2002 study is the comparision of this risk group assessment to the MRD-based criteria used in ALL-BFM 2000. Methods. MRD in BM and PB samples was assessed by patient-specific RQ-PCR for clonal immunoglobulin and T-cell receptor (ig/TcR) gene rearrangements. Results. In total 184 patients treated according to ALL IC-BFM 2002 in the Czech Republic, Israel, Hong Kong and Uruguay were investigated for the presence of clonal ig/TcR rearrangements. At least one patient-specific RQ-PCR target with minimal sensitivity of 10(-4) was designed for 161 patients. In these patients, MRD in BM at several time-points of therapy (including mandatory points at weeks 5 and 12) was evaluated; the PB specimens of Czech T-ALL patients were tested simultaneously. In total, 621 follow-up BM specimens and 50 PB samples were tested. The results showed separation of MRD levels between standard-risk group (SRG) and intermediate-risk group (IRG) stratified patients at day 33 (p=0.005). However, in 21 of 66 SRG patients (31.8%), MRD positivity at week 5 and/or at week 12 was observed (ranging from the positivity below QR to 1.5x10(-21) to 1.5x10(-21)), thus identifying patients who would not qualify to the MRD-based SRG in ALL-BFM 2000 despite the identical induction regimen. Conversely, 24% of IRG patients showed MRD negativity by two independent ig/TcR targets at both critical time-points, thus accomplishing the ALL-BFM 2000 SRG criterion. As expected, high-risk group (HRG) patients showed significantly slower molecular response than other groups. Taken together, patients with BCP ALL had significantly lower MRD levels at day 15 (p=0.0004) and at day 33 (p=0.0004) than T-ALL patients. There was no significant difference in MRD levels between the two groups at week 12. In 80 follow-up T-ALL samples, MRD levels in PB clearly paralleled those in BM.

Conclusions. Our findings revealed a significant difference between the stratification results of ALL IC-BFM 2002 and ALL-BFM 2000. Fast morphological response to treatment (M1 or M2 bone marrow at day 15) together with other low-risk features does not necessarily correspond with rapid MRD clearance.

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MULTIVARIATE ANALYSIS INCLUDING MTHFR GENOTYPES IN A COHORT OF ALL PATIENTS


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Background. Several prognostic factors have been used to stratify ALL patients' risk. These prognostic factors include clinical and biological characteristics (age, WBC count, cytogenetic or molecular aberration and most recently the kind of early response to treatment). Recently the influence of polymorphisms of different genes involved in metabolism of chemotherapeutic agents have been studied especially in childhood ALL. Methylenetetrahydrofolate reductase (MTHFR) catalyzes conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate in the folic

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acid cycle. The C677T and A1298C MTHFR polymorphisms affect MTHFR enzyme causing a reduction of its activity, altered distribution of intracellular folate metabolites. Since MTHFR plays this important role in folate metabolism, differences in its activity due to these two gene variants might modulate therapeutic response to antifolate chemotherapeutic agents. In this study we evaluated the influence of classical prognostic factors and of C677T and A1298C MTHFR polymorphisms on time to relapse and survival in a group of 82 ALL patients. Patients. Patients’ characteristics were: 46/36 M/F; median age 37.5 (12-75), B-phenotype L1-2 64pts, L3 7pts and T phenotype 11, normal karyotype in 45pts and high risk karyotype in 28pts. Forty-seven patients showed WBC>10x10^9/L at diagnosis. Forty-four pts relapsed at a median follow-up of 12 months (range 1-159) and 34 pts are alive at a median follow-up of 21 months (range 1-190). Results. The genotypes frequencies were consistent with previous published reports. The polymorphisms’ distribution among different karyotype groups was homogeneous. On univariate analysis, pts with the MTHFR C677T and A1298C variant alleles did not experience significantly increased relapse and mortality risk (chi-square test p=0.82 and p=0.59 for 677 and p=0.36 and p=0.72 for 1298). Comparison of RFS and EFS between homozygous wild type and variant patients in both 677 and 1298 polymorphisms was not significantly different. The Cox regression analysis containing gender, age, WBC, karyotype, phenotype and MTHFR genotypes showed an increased hazard ratio (HR) relapse and mortality in patients with high risk karyotype (p<0.001 and HR 4.33 and p=0.79, p=0.3, p=0.57 respectively), while RFS and EFS were significantly decreased in the presence of high risk karyotype and age >24y (HR 4.33 and p=0.0001 and p=0.15 respectively). The Cox regression analysis containing gender, age, WBC, karyotype, phenotype and MTHFR genotypes showed an increased hazard ratio (HR) relapse and mortality in patients with high risk karyotype (p<0.001 and HR 4.33 and p=0.79, p=0.3, p=0.57 respectively), an increased HR mortality calculated in pts older than 24 years (p<0.001 and HR 4.15). Regarding WBC count at diagnosis there was no significant correlation between WBC>10x10^9/L and outcome whilst we found an increased risk of mortality among patients with WBC>5x10^9/L (chi-square test p=0.006). Conclusions. In our study we did not observe any association between MTHFR polymorphisms and relapse and survival rate in a group of almost adult ALL patients. Our data are in contrast with those from other groups which evaluated the influence of these two polymorphisms in pediatric standard risk patients. Due to the higher frequency of molecular alterations (9,22 and t4;11) in our context MTHFR polymorphisms per se has not enough power to influence DFS and EFS, when compared to classical risk factors like karyotype aberrations, WBC at diagnosis and age influencing prognosis.

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DASATINIB IN PATIENTS WITH CHRONIC MYELOGENOUS LEUKEMIA IN LYMPHOID BLAST CRISIS OR PHILADELPHIA CHROMOSOME POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA THAT IS IMATINIB-RESISTANT OR INTOLERANT: THE CAL008015 START-L STUDY

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Background. Dasatinib (D) (BMS-354825) is a multikinase inhibitor of Bcr-Abl and Src. In a phase I study, hematologic responses were achieved in D in pts with LB-CML and Ph+ ALL. Aims. To estimate the major hematologic response (MaHR) rates to D in IM-R and IM-1 patients with LB-CML and Ph+ALL. Methods. START L is an open label phase II study of D in IM-R or IM-1 pts with LB-CML and Ph+ALL which was conducted in 42 centers worldwide. D was given orally, 70 mg twice a day (bid), with escalation to 100 mg bid for inadequate initial response or reduction to 50 mg and 40 mg bid for toxicity. Pts had weekly blood counts and monthly bone marrow (BM) exams, including cytogenetics. Molecular evaluation of Bcr-Abt transcripts was performed by real-time quantitative PCR at baseline and at complete cytogenetic response. Bcr-Abl measurement was assessed at time of progression and at time of baseline. The primary endpoint was confirmed (sustained for at least 4 weeks) major hematologic response rates (MaHR). Results. From January to June 2005, 94 pts were treated. Data are available on the first 42 LB-CML and 36 Ph+ ALL treated pts. Of the 48 LB-CML pts, 42 had IM-R, 52% were male and median age 47 yrs (range 15-85). 40 pts had IM-1, 52% were male and median age 47 yrs (range 19-85). Prior therapy included IM>600 mg/day in 52% pts and stem-cell transplant (SCT) in 31% pts. Median baseline platelet (plt) count was 351/L, median BM blasts was 82%. Of the 46 Ph+ALL pts, 44 were IM-R, 59% were male with median age of 47 yrs (range 15-85). Prior therapy included IM>600 mg/day in 45% pts and SCT in 37% pts. Baseline plt count was 62/L, median BM blasts were 70%. Preliminary efficacy and safety analyses are currently available on the first 78 pts. Among the 42 LB-CML pts, the D dose was escalated in 14%, temporarily interrupted in 33%, and escalated in 26% of pts. At 6 months, the MaHR rate was 31% including 26% complete hematologic response (CHR), the MCyR rate was 50%, and 17% pts remained on study. Among the 36 Ph+ALL pts the D dose was reduced in 28%, temporarily interrupted in 39%, and escalated in 47% of the pts. At 6 months, the MaHR rate was 42% including 31% CHR, the MCyR rate was 55%, and 33% pts remained on study. Among all 78 pts, grade 3-4 thrombocytopenia and neutropenia were seen in 82% and 76% of pts, respectively. The most frequent D-related non-hematologic toxicities were diarrhea (80%), nausea (28%), fatigue (19%), rash (17%) and pleural effusion (13%). Conclusion. D has substantial activity in heavily pretreated LB-CML and Ph+ ALL pts. Data on all 94 pts will be presented at the meeting including an analysis of the molecular response and Bcr-Abl mutations.

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CRYPTIC KARYOTYPE DEFECTS ARE DISCOVERED BY INTERPHASE FLUORESCENCE IN SITU HYBRIDIZATION (I-FISH) IN CHROMOSOMALLY NORMAL ADULT B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA (B-ALL).


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In adult B-ALL the chromosome pattern plays a pivotal role in the prognostic stratification of patients and in driving therapeutic decisions. Unfortunately, conventional cytogenetics (CC) is not always informative since it is often hampered by either the absence of mitotic cells or by the bad quality of metaphases. Thus, FISH, which can be performed on mitotic as well as on quiescent cells, has progressively been used in addition to CC to unmask chromosomal changes and cryptic defects in B-ALL. We have applied I-FISH to analyse 58 adult B-ALLs who showed a normal karyotype pattern on CC and G- and Q-banded metaphases. Our study was aimed at establishing the true incidence of BCR-ABL, ETV6-AML1, MLL rearrangements and p16/INK4A deletions in chromosomally normal B-ALL and at correlating our findings with clinical parameters and outcomes. The 58 patients examined, 22 females and 16 males with a median age of 41 years (range 16-78), were part of a large series of 263 consecutive adult B-ALL patients referred to our observation during the period (1994-2005). Within this series 56 patients (21.2%) did not yield metaphases and 73 (27.7%) presented a normal chromosome pattern. I-FISH was carried out with the following commercial probes: LSI BCR/ABL1 dual color single fusion, LSITEL/AML1 ES, LSI MILL and LSI MLL dual color single fusion, LSI ETV6-AML1 ES, LSI CEP 9 dual color single fusion, LSI ETV6 and LSI 9p21. Hybridization procedures were carried out according to manufacturers’ guidelines. Cut-off values were determined after having analysed two-hundred cells from ten normal controls and using a one-sided binomial distribution with a 95% confidence interval. So, the cut-off values were fixed at 10% and 6% for the BCR/ABL1 and MLL probes and at 3% for both the ETV6-AML1 and the LSI p16 (9p21)/CEP 9 probes. I-FISH discovered clonal chromosome defects in a total of 17/38 (44.7%) patients. The loss of either one or two red spots corresponding to the LSI p16 (9p21)/CEP 9 probe was the most common cryptic abnormality, being observed in 10 patients (26.3%). No patient presented a cryptic BCR-ABL or ETV6-AML1 rearrangement. The amplification of the AML1 gene and the monosomy of the ETv6 gene were observed in 11-15% cells from 5 and 2 patients, while the monosomy and the amplification of the MLL gene in one patient each. The 2 patients with p16 nullisomy were unresponsive to chemotherapy and survived four and six months; those with p16 monosomy were too few to obtain any prognostic information. Conclusion. i) I-FISH in combination with CC is a very useful tool to unmask cryptic defects in B-ALL since it readily discovered genetic aberrations in 44% of our chromosomally normal patients, ii) the p16/INK4a monosomy is a very common clonal defect not only in T-ALL but also in B-ALL, iii) ETV6-AML1 and MLL rearrangements are extremely rare in adult B-ALL, iii) CC is very effective in detecting Ph positive cells although the clonal cell population is more accurately defined by I-FISH.

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11th Congress of the European Hematology Association
WILMS TUMOR GENE 1 EXPRESSION IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. Wilms’ tumor gene 1 (WT1), located on chromosome 11p13, encodes a zinc-finger transcription factor with important roles in embryogenesis and oncogenesis. WT1 is supposed to be overexpressed in the majority (70-90%) of acute leukemias and has been identified as an independent prognostic factor. However, results on the characteristic and limited number of samples and controls regarding WT1 expression and its clinical impact become controversial. Many of the discrepancies could be attributed to the non-standardized techniques of WT1 detection and quantification, different patients’ characteristics and limited number of samples and controls investigated. Objectives. The aim of this study was to establish reproducible PCR assays for WT1 detection and evaluate WT1 expression in a representative group of childhood ALL patients. Methods. RT-PCR and Q-PCR enabling absolute quantification of total WT1 and its four main isoforms (variants A, B, C and D) were designed, optimized and validated according to BIOMED-1 Concerted Action [van Dongen et al., Leukemia 1999] and Europe Against Cancer Program [Gabert et al., Leukemia 2005] recommendations, respectively. With these methods we evaluated WT1 in diagnostic bone marrow (BM) samples of 125 consecutively enrolled childhood ALL patients (106 B-ALL, 19 T-ALL); normal peripheral blood (PB) and BM, and regenerating MRD negative BM were used as controls. Results. Low WT1 expression of a uniform pattern was present in all control samples. In BCP-ALL, we detected a wide range of WT1 levels (5 logs) with median close to that of normal BM; in T-ALL, WT1 expression in T-ALL was significantly higher (p<0.001). Patients with MLL-AF4 translocation showed considerable WT1 overexpression (p<0.01) compared to other patients. Older children expressed higher WT1 levels than children under 10 years of age (p<0.001), while there was no difference between patients with WBC over 50×10⁹/L and lower. There was also no correlation between WT1 and CD34 expression. Analysis of relapsed cases (14/125) indicated that abnormal increase or decrease in WT1 expression was associated with significantly increased risk of relapse (p=0.0006), and this prognostic impact of WT1 was independent of other main risk factors (p=0.0012). All four WT1 isoforms were detected in normal controls and ALL samples. Preliminary results did not show a significant difference in WT1/exon5+[+ and WT1/KTS][+ ratio in ALL patients compared to normal BM. Conclusion. In summary, WT1 expression in childhood ALL is variable and much lower than in AML or adult ALL. WT1 thus will not be a useful marker for MRD detection in childhood ALL, however, it does represent a potential independent risk factor in childhood ALL. Interestingly, WT1 expression in childhood ALL patients expressing WT1 at levels below the normal physiological bone marrow WT1 expression, and this reduced WT1 expression also appears to be associated with a higher risk of relapse. The designed RT-PCR and Q-PCR assays for detection of WT1 and its main isoforms could be considered as standards for future reference and use.

CELL BIOLOGICAL FEATURES OF BLASTS PERSISTING AT DAY 8 OF INDUCTION THERAPY IN CHILDHOOD PRECURSOR B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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In childhood acute lymphoblastic leukemia (ALL), persistence of leukemic blasts during therapy is of crucial prognostic significance. In the frontline ALL-BFM (Berlin-Frankfurt-Münster) trial, treatment stratification is based on blast count estimation in peripheral blood at day 8 of induction prephase with prednisone and one intrathecal dose of methotrexate. Recently, we investigated genome-wide gene expression of blasts persisting after one week of induction therapy (day 8 blasts). The observed expression changes in day 8 blasts as compared with blasts at initial diagnosis (day 0 blasts) included key regulators of the cell cycle and genes encoding for B-cell differentiation markers. Furthermore, we observed early induction increase of inflammatory response genes and a decrease of BCL2, the prototypic member of the anti-apoptotic BCL2-subfamily. In the current study we analyzed day 8 blasts at protein and cellular levels. Firstly, we isolated the day 0 and day 8 blasts of 13 patients by flow sorting and measured the cell cycle distributions at both days. As a result, mean percentage of cycling (S, G2/M-phases) cells in the blast subpopulations significantly decreased from 5.1% (range: 0.2-22%) at day 0 to 1.2% (range: 0.1-5.1%) at day 8 (p=0.014). In a total series of 56 patients, flow cytometric analysis confirmed expression changes of the B-cell differentiation markers CD10 (decrease by 1.4-fold), CD20 (increase by 2.4-fold), CD54 (decrease by 1.3-fold) and TdT (decrease by 2.4-fold) (p<0.005). Moreover, we were also able to confirm the expression increase of the inflammatory response molecules CD11b (5.1-fold, p=0.001, n=15) and IFNGR (2.2-fold, p<0.001, n=15), and the decrease of the BCL2 protein (1.5-fold, p<0.001, n=29). Taken together, the cell biological characterization of ALL cells persisting during induction therapy demonstrated an inhibited cell proliferation and an overall gene expression shift towards resting mature B cells. Furthermore, expression decrease of BCL2 in the day 8 blasts points to the involvement of this anti-apoptotic protein in the molecular mechanism of action of glucocorticoids in childhood ALL.

MINIMAL RESIDUAL DISEASE MONITORING OF BCR-ABL POSITIVE ADULT ACUTE LYMPHOBLASTIC LEUKAEMIA USING QUANTITATIVE REAL-TIME PCR: PRELIMINARY RESULTS FROM THE MRC-UKALLXII TRIAL

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Background. Adult ALL carrying the BCR-ABL fusion-gene is associated with a dismal prognosis. Minimal residual disease (MRD) is a significant tool for monitoring disease progression and outcome and BCR-ABL offers a convenient target for molecular MRD monitoring by QRT-PCR. In 2003, the MRC UKALLXII trial was amended to incorporate the addition of the tyrosine-kinase inhibitor Imatinib Mesylate for BCR-ABL+ patients during intensification. 25-30% of adult ALL patients exhibit BCR-ABL and the p190BCR-ABL isoform is more common than the p210BCR-ABL. It remains to be seen whether isoform type carries clinical significance. Aims. To investigate the efficacy of monitoring response to induction therapy and Imatinib in adult ALL patients enrolled on the MRC-UKALLXII trial using BCR-ABL as a target for molecular MRD. Methods. MRD analysis was performed using Roche LightCycler 1.2 with SYBR-Green fluorescent technology and primers for ABL, BCR, and CD34 expression. Analysis of relapsed cases (14/125) indicated that abnormal increase or decrease in WT1 expression was associated with significantly increased risk of relapse (p=0.0006), and this prognostic impact of WT1 was independent of other main risk factors (p=0.0012). All four WT1 isoforms were detected in normal controls and ALL samples. Preliminary results did not show a significant difference in WT1/exon5+[+ and WT1/KTS][+ ratio in ALL patients compared to normal BM. Conclusion. In summary, WT1 expression in childhood ALL is variable and much lower than in AML or adult ALL. WT1 thus will not be a useful marker for MRD detection in childhood ALL, however, it does represent a potential independent risk factor in childhood ALL. Interestingly, WT1 expression in childhood ALL patients expresses WT1 at levels below the normal physiological bone marrow WT1 expression, and this reduced WT1 expression also appears to be associated with a higher risk of relapse. The designed RT-PCR and Q-PCR assays for detection of WT1 and its main isoforms could be considered as standards for future reference and use.
SHOULD HYPERCVAD / METHOTREXATE-CYTARABINE BE CONSIDERED A STANDARD TREATMENT IN ACUTE LYMPHOBlastic LEUKEMIA? A BRAZILIAN EXPERIENCE

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Background. Adult ALL is traditionally treated by a Vincristine and Prednisone protocol with the addition of an Anthracycline. Early dose intensification as described by the MD Anderson group with the hyperfractured and intensified Cytoxan, Vincristine, Dexamethasone/hight dose methotrexate-cytarabine regimen has shown in there experience an improvement in disease free survival. There are few data about the experience with this high dose protocol from other groups.

Methods and Results. We analysed retrospectively 65 patients treated between 1994 and 2005 in 3 brazilian hospitals. Median age was 21 years; only 3 patients were > 60 years. The incidence of Philadelphia positive ALL was 6% and T-ALL 23%. According to age, leukocyte count, SNC disease at diagnosis, remission after first cycle, patients were classified as high risk (69.5%) or low risk (30.5%). Overall 54 (80%) patients achieved complete remission after the first chemotherapy cycle. The median time between treatment cycles was 56 days (range 27-83) mainly because of severe infections. Recurrence of the disease occurred in 5 patients (7%) during induction. Forty patients (61%) were able to complete the 8 induction courses. Twenty three (33%) patients died during induction. The better stratification (especially neurological), who were responsible for the high rate of early deaths. The occurrence of severe infections and toxicities, was found in 26/89 cases (29.2%). A significant correlation (p<0.010) was observed between p21 protein expression and induction deaths (33%), also results in a delay in treatment and consequently in relapse of the disease. Several patients had to interrupt the protocol and start another treatment regimen. The better stratification of patients, the use of a less toxic induction regimen and subsequent risk adapted intensification, seems in our view a better approach for ALL patients.

IMATINIB COMBINED TO INDUCTION OR CONSOLIDATION CHEMOTHERAPY IN YOUNGER PATIENTS WITH DE NOVO PHILADELPHIA CHROMOSOME-POSITIVE ACUTE LYMPHOBlastic LEUKEMIA RESULTS OF THE GRAAPH-2003 STUDY


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Combination of imatinib with standard chemotherapy has been recently reported as very promising in patients with Ph+ ALL. Between January 2004 and October 2005, 45 patients with newly-diagnosed Ph+ ALL (median age, 45 years; median follow-up, 11 months) were treated in the GRAAPH-2003 study in which imatinib was started with HAM consolidation in good early responders (cortico- and chemo-sensitive ALL) or earlier during the induction course in combination with dexamethasone and vincristine (DIV regimen) in poor early responders (cortico- and/or chemo-resistant ALL). In all patients, imatinib was then continuously administered until stem cell transplantation (SCT), either allogeneic if aged 55 years or less with a matched familial or unrelated donor, or autologous if older or no donor but in molecular remission. Overall, hematological and molecular remission rates were 96% and 62%, respectively. The rate of patients able to receive SCT as planned by the protocol was 65%. At 18 months, estimated disease-free and overall survival was 51% and 65%, respectively. All these endpoints compared very favorably with results obtained in the pre-imatinib LALA-94 trial. Interestingly, the bad prognosis previously associated with a poor early response to standard therapy, a lack of allogeneic donor, and a non-achievement of molecular remission was not evidenced in this study. In conclusion, this study confirms the value of the combined approach in younger patients with Ph+ ALL and encourages prospective trials to define the optimal chemotherapy which has to be combined with ima- tinib and carefully re-evaluate the place of allogeneic SCT in this new context.

ROLE OF P21 IN ADULT ACUTE LYMPHOBlastic LEUKEMIA PATIENTS: METHYLATION STATUS AND PROTEIN EXPRESSION.


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Background. Methylation of Cpg islands in the 5’ gene region is associated with transcriptional silencing of gene expression. The hypermethylation of tumor suppressor genes has been described in gastric and pancreatic cancer, as well as in acute myeloid leukemia, suggesting its potential role in neoplasia. Among the three members of the Kip/Cip family of cyclin dependent kinase inhibitors (CDK) p21, p27 and p57, little is known about their methylation status in hematological malignancies. Contrasting studies have been reported on the role of p21 hypermethylation in acute lymphoblastic leukemia (ALL). Aims. To analyze p21 gene methylation status and protein expression in primary blasts from adult ALL enrolled in the GIMEMA protocol LAL2000. Methods. Human leukemic cell lines, normal peripheral blood lymphocytes (PBL) and 89 primary samples from untreated ALL patients were evaluated in this study. The p21 gene methylation status was investigated using a widely accepted method based on bisulfite modification of DNA, followed by the use of methylation-specific PCR assay (MS-PCR). This assay was further validated in vitro by SSI methylase. The p21 protein expression was analyzed by Western blot using the p21-WAF1/MoAb (Santa Cruz, CA). Results. The human lymphoblastic cell lines RPMI8866 and CEM, the myeloid cell line OCI-AML3 and normal PBL from 10 healthy donors were characterized by a consistent p21 promoter unmethylation (negative controls). In contrast, a weak methylation was documented in the Raji and Jurkat cell lines, while the Racl (Burkitt’s lymphoma) cell line was strongly methylated (positive controls). In addition, p21 protein expression was found in the OCI-AML3, Raji and RPMI8866 cell lines, while it proved negative in the Jurkat and Racl cell lines, and in normal PBL. Sixty primary ALL cases evaluated for p21 methylation status showed a consistent unmethylation in all samples, while the p21 protein expression was found in 26/89 cases (29.2%). A significant correlation (p<0.010) was observed between p21 protein expression and immunophenotype, 37.5% of B lineage ALL compared to 8.3% of T lineage ALL. In addition, a trend was found between p21 expression and age. Achievement of CR was observed in 65.4% and 79.4% of p21 positive and negative cases, respectively. Summary. While p21 gene methylation does not appear to play a pathogenetic mechanism in adult ALL, p21 protein expression is found in one third of these patients suggesting a role in the disease.
PROGNOSTIC VALUE OF HOX11L2/TLX3 AND TAL1/SCL EXPRESSION IN CHILDHOOD T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA: RESULTS OF THE FRALLE 93 PROTOCOL

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Background and aim of the study. The most frequent oncogenic activation events characterized in childhood T acute lymphoblastic leukemia (T-ALL) result in the transcriptional activation of genes coding for transcription factors. The main genes are TAL1/SCL, a member of the basic region helix-loop-helix gene family, HOX11L2/TLX3 a member of the tal1 region helix-loop-helix gene family, HOX11L2/TLX3 a member of the tal1 region helix-loop-helix gene family, HOX11L2+ and none of these were respecti...
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MATURE B-CELL ACUTE LYMPHOBLASTIC LEUKAEMIA: A REPORT OF 31 PEDIATRIC CASES

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Background and Aim. The cure rate for childhood acute lymphoblastic leukemia (ALL) now exceeds 75%. This rate has been achieved in part by improvements in the biologic characterization of newly diagnosed patients. The stage of maturation of leukemic cells, as defined by immunologic markers, has been shown to be strongly associated with treatment outcome in B-lineage childhood ALL. To demonstrate the clinical significance of a B mature phenotype, we studied the presenting immunophenotyping and a mature B-cell phenotype were compared with those of the entire pediatric ALL population. Results. Thirty-one children with mature B-ALL diagnosed between 1989 and 2004 from eleven centers of the EORTC-CLG have been included in the study. All patients had blasts that express at diagnosis surface immunoglobulins (sIg) and a morphologic presentation of a mature B-cell phenotype. Patients and Methods. Thirty-one children with mature B-ALL diagnosed between 1989 and 2004 from eleven centers of the EORTC-CLG have been included in the study. All patients had blasts that express at diagnosis surface immunoglobulins (sIg) and a morphologic presentation of a mature B-cell phenotype. We studied the clinical and biological characteristics and survival of 31 children with this phenotype. Patients and Methods. Thirty-one children with mature B-ALL diagnosed between 1989 and 2004 from eleven centers of the EORTC-CLG have been included in the study. All patients had blasts that express at diagnosis surface immunoglobulins (sIg) and a morphologic presentation of a mature B-cell phenotype. We studied the clinical and biological characteristics and survival of 31 children with this phenotype.

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EFFICACY AND TOXICITY OF IDA/NOVA-FLAG REGIMEN AS SALVAGE TREATMENT FOR PATIENTS WITH ACUTE LEUKAEMIA FIVE-YEAR-EXPERIENCE OF A SINGLE INSTITUTE

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Background. The observation that Fludarabine administration before Arcaricine can lead to larger cytoplasmic concentrations of the latter, was the origin for the design of IdA/Novo-FLAG regimen. Aim. The present study aims to show that the IdA/Novo-FLAG regimen is a safe and efficacious treatment option for patients with Acute Leukemia (AL) including elderly patients or relapsed AL-M. Methods. During the last five years 44 pts (32 pts<65 year-old and 12 pts≥65 year-old) with AL received in our department the IdA/Novo-FLAG regimen. Their disease was either primary resistant to chemotherapy (17%) or relapsed during therapy (21%) or within the first year after completion (5). The chemotherapeutic protocol included: Fludara- bine: 25mg/m² in 1h iv infusion, 4 hours later Arcaricine:3 g/m² (or 1g/m² for pts< 65 years of age) in 3h iv infusion and 1 hour later Idarubicin: 12mg/m² or Novantrone: 10mg/m² 1/2 h iv infusion. Results. Myelosuppression with febrile neutropenia (55% of episodes with microbiological documentation) and the other unspecified-FUO was the main toxicity of the regimen. Treatment related mortality (TRM) was 22%, 5%. The incidence of TRM was 50% among older pts and 20% among pts<65. Six pts died due to infection and in 4 due to hemorrhage. Two pts > 65 (16%) and17 pts< 65 (55%) obtained CR after IdA/Novo-FLAG. Eight pts with primary resistant disease (44,4%), 7 pts who relapsed during therapy (33%) and 4 pts who relapsed shortly after it (80%) were in CR after IdA/Novo-FLAG. The following table shows the response according to type of disease.

Table 1.

<table>
<thead>
<tr>
<th>Disease/number of pts</th>
<th>CR</th>
<th>PR</th>
<th>SD</th>
<th>PD</th>
<th>Early Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALL (N=15)</td>
<td>8</td>
<td>53.3%</td>
<td>2</td>
<td>13 (3%)</td>
<td>3 (20%)</td>
</tr>
<tr>
<td>De Novo AML (N=16)</td>
<td>7</td>
<td>43.7%</td>
<td>2</td>
<td>12.5%</td>
<td>5 (31.2%)</td>
</tr>
<tr>
<td>AML from preexisting MDS (N=12)</td>
<td>2 (16.6%)</td>
<td>1</td>
<td>8.3%</td>
<td>5 (41.6%)</td>
<td>3 (25%)</td>
</tr>
<tr>
<td>SUM (N=44)</td>
<td>17</td>
<td>38.6%</td>
<td>5</td>
<td>11.4%</td>
<td>3 (29.5%)</td>
</tr>
</tbody>
</table>
The median duration of neutrophil (>500/µl) and platelet (>50,000/µl) recovery was 18 days (min: 11-month, max: 75-months). The TTP for pts who achieved CR was 2.5 months. Five pts (11%) achieved allo-BMT after Ida/Nova-FLAG, however 4 experienced relapse within the first trimester after transplantation. 13 pts received third line treatment after Ida/Nova-FLAG (e.g. Mylotarg, L-asparaginase, Hycamptin-Ara-C, and ESHAP) but only 1 obtained CR and the other did not respond. Conclusion: The state of immunosuppression in children with ALL or NHL presents acceptable toxicity and favorable outcome, even for those with refractory ALL, for whom the data in the literature is sparse. Finally Ida/Nova-FLAG regimen can be a treatment modality for allo-BMT candidates.

**0667**

**EFFECT OF INTENSIVE CHEMOTHERAPY ON INNATE IMMUNITY IN CHILDREN WITH ACUTE LEUKEMIA AND NON-HODGKIN LYMPHOMA**

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Background. Intensive combination chemotherapy in acute leukemia (AL) and non-Hodgkin lymphoma (NHL) results in a profound systemic immunosuppression. This state, in some patients, may be responsible for recurrent and sometimes life-threatening infections. Objective. The aim of this study was to examine the recovery of three cell subpopulations: neutrophils and macrophages in peripheral blood of children with ALL and NHL after an intensive chemotherapy. Patients and Methods. The study group consisted of 27 children (18 patients with acute lymphoblastic leukemia (ALL), 3 children with acute myelogenous leukemia (AML) and 6 NHL patients), aged 3 to 16 years, treated in the Department of Pediatrics, Hematology, Oncology and Endocrinology at Medical University of Gdansk. Each patient was examined 2 weeks after cessation of an intensive chemotherapy and thereafter every three months during a year. The study consisted of a medical examination, anaemia towards infections and laboratory tests. The whole blood count, the lymphocytes subpopulations (NK cells CD3-CD16+CD56+ and the non-specific immunosuppressive T cells CD3+CD8high+CD57+) were analyzed with flow cytometry. NK cytotoxic activity was measured with colometric assay based on cytoplasmatic LDH activity released by damaged cells. The investigation of phagocytosis was performed by flow cytometry (ingestion of HTCC labelled opsonized E. coli bacteria by granulocytes and monocytes in whole blood was measured). Results. The results of our investigation indicate that: 1. The state of immunosuppression such as leucopenia remained in the patients stable during the observation time; 2. There was a noticeable tendency towards a decrease with subsequent rapid increase of cytotoxic NK response with a stable percentage and absolute number of NK cells and the immunosuppressive T cells subset; 3. The phagocytic activity of neutrophils was increased at the beginning of observations and three months thereafter it started to decrease; 4. The phagocytic activity of monocytes remained stable during the whole period of observation. Conclusions. Intensive chemotherapy in children with acute leukemia and NHL besides severe leucopenia induces also transient changes in cytotoxic function of NK cells and phagocytic activity of neutrophils.

**0668**

**RISK-ADAPTED THERAPY FOR ELDERLY PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA**

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Background. Acute Lymphoblastic Leukemia is uncommon and scantily curable in patients over 60 years of age because of a greater resistance to chemotherapy, a relative inability of elderly patients to face the toxic effects and complications of therapy and influence of co-morbidities. Aims: We review here our experience of 44 consecutive cases of ALL of elderly age collected in the last fifteen years. Median age was 66 years (range 61-85); 12/11 FAB classification: 3/6; Median WBC was 15×109/L (range 1-80); Male/Female ratio was 26/18. Forty cases (90.9%) belonged to B cell lineage (pre-pre-B 11, common 24, pre-B 5) and 4 (9.1%) to T cell lineage (pre-T stage); CD34 expression was observed in 27/34 cases (79.4%); CD3, CD13 and CD15 surface expression was positive in 17/35 (48.6%), 14/34 (41.2%) and 5/24 cases (20.8%), respectively; overall, CD13 and CD34 were co-expressed on 9/34 cases (26.5%). Philadelphia chromosome was present in 13 patients (29.5%). Methods. Out of the 44 revisited patients, 31 younger patients (median age 65 years, range 61-77, good performance status and without co-morbidity factors), received an intensive treatment such as LAL and IMAGema protocols. In the remaining 13 elderly patients (median age 77 years (range 61-83) and those with severe co-existing cardiac, pulmonary, renal and hepatic disease, a gentle chemotherapy including prednisone and vincristine, 6-mercaptopurine and methotrexate was utilized. Results. Six patients (19.3%) of the group treated with curative intent died during the induction phase; 19 patients (61.3%) achieved a CR and, at present, 3 patients are alive +10, +46 and +105 months. Out of 13 patients receiving less intensive and supportive treatment, only 4 (30.8%) achieved a short CR: all the patients had an early relapse and died after 4, 5, 6 and 12 months. Conclusion. Our data demonstrate that immunophenotypic and karyotypic patterns of these patients differ from those usually observed in children and adults with ALL, therefore confirming the presence of a stem cell disorder and an extremely poor prognosis. In addition, in our experience emerged that to the ‘biologically younger patients’ who can well tolerate an aggressive therapy this approach should not be denied because of it is possible to achieve longer survivals.

**0669**

**PROGNOSTIC RELEVANCE OF THE IMMUNOPHENOTYPE IN ADULTS AND CHILDREN WITH T-LINEAGE ACUTE LYMPHOBLASTIC LEUKEMIA**

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Background. T-lineage Acute Lymphoblastic Leukemia (T-ALL) accounts for 15-20% of newly diagnosed cases of ALL. It is characterized by a male predominance, high WBC count, mediastinal tumors and central nervous system involvement. Historically, T-ALL patients (pts) have a worse prognosis than other ALL patients. Aims. In this paper we review our experience on 66 consecutive pts with T-ALL (17 children and 49 adults) diagnosed and treated in our center. Median age of adults and children was 22 (range: 16-75) and 9 (range: 4-15) years, respectively. Male/Female ratio was 47/19 (adults 33/16; children 14/3) Methods and Results. Based on their immunophenotype, all pts were classified in 3 ontogenic stage-related subtypes: 1. Early T-ALL (immunophenotype: CyCD3+/CyCD7+/CyCD1-/CyCD34-); 39 pts (59,1%) belonged to this group. 2. Intermediate T-ALL (immunophenotype: CyCD7+/CyCD3+/CyCD34-); in 14 pts (59,1%) a mediastinal mass was present; CD34 expression was observed in 26/34 cases (76,5%); myeloid antigens (MyAg) (CD13 and/or CD33 and/or CD15 and/or CD65) were co-expressed in 18/35 cases (51,4%). 3. Cortical T-ALL (immunophenotype: CyCD7+/CyCD1+/CyCD34-); 20 pts (50,5%) were included. Adult/Childhood ratio was 12/8; Median WBC was 89×109/L (range 7-1000); mediastinal tumor was present in 13 pts (65%); CD34 was positive in 4/17 cases (23,5%) and MyAg were co-expressed in 1/16 cases (6,2%). III. Mature T-ALL (immunophenotype: CyCD7+/CyCD1-/CyCD34-): the remaining 7 pts (10,6%) were included. Adult/Childhood ratio was 4/3; Median WBC was 10×109/L (range 4-480); mediastinal tumor was present in 4 pts (57,1%); none of them expressed CD34 and MyAg co-expression was only present in one case (14,3%). Therapeutic approaches applied during the twenty years period of the study were those of GIEMA (for adults) and AIEOP (for children) cooperative groups. Overall, 51 pts (77,5%) achieved Complete Remission: (55 (71,4%) and 16 (94,1%) for adults and children, respectively). Considering the patients’ age, 29 (62,2%) early T-ALL, 18 (90%) cortical T-ALL and 6 (57,8%) mature T-ALL pts significantly achieved CR (p=0.035); of these, at present (median follow-up 136 months - range: 5-236), 24 pts are alive in CCR (subtype I: 10 patients (57%); subtype II: 12 pts (66,7%); subtype III: 2 pts (55,5%) p=0.012). Conclusion. Our data confirm that T-ALL may be quite heterogeneous in terms of clinical and biological features; a lower incidence of lymphomatous features was observed in the less mature subtypes of T-ALL, in which, in contrast, an higher co-expression of CD34 and MyAg was found. From our experience comes out that
the immunologic classification is the most significant prognostic factor in T-ALL; in fact, in adult as well as in children T-ALL, the cortical subtype showed a better outcome as compared to early and mature subtype.

**0670**

**BONE MINERAL DENSITY IN SURVIVORS OF ACUTE LYMPHOBLASTIC LEUKEMIA DURING CHILDHOOD**

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Cure rates for children with ALL now approach 80%. Therefore, the late adverse effects of chemotherapy are more frequently observed. These children are especially at high risk of developing low bone mineral density (BMD) predisposing to severe osteoporosis in adulthood. The aim of this study was to evaluate BMD and bone mineral metabolism (BMM) and the influencing factors on them. Method: We analyzed the data of 70 children who achieved complete remission with ALL-BFM protocols. Their median follow up period was 4.3 years. Children were treated according to their leukemia risk specific groups (Standard:16, Median:45, High:9). The groups according to cessation of treatment included within one year, between 1-2 years and longer than 2 years. Their height and weight measurements and percentiles were determined both at the time of diagnosis and when they were included into the study. BMD at post-chemotherapy was also measured in lumbar area with dual X-ray absorptiometry (DEXA), and the results were expressed as age and sex-specific z scores. Serum IGF-1 and 25(OH) D-vitamin levels were measured at the time of study and the results were compared with the healthy controls. Using logistic regression test, we compared the association of BMD change with the cessation of treatment, risk groups, the cumulative steroid dose, cranial radiotherapy, passive smoking, duration of television watching and daily calcium intake. Serum IGF-1 and 25(OH) D-vitamin levels in each risk group were compared. Logistic regression analyses revealed that the most significant factor influencing BMD was daily calcium intake (OR: 0.997; 95%CI: 0.995-0.999). Results. The mean age of children at the time of diagnosis and study were 5.7±3.4 and 10.6±3.8 years, respectively. Percentiles both for height and weight at diagnosis and postchemotherapy increased non-significantly. The mean BMD and z score were found 0.602±0.15 g/cm² and -1.72±1.05, respectively at the time of study. The increase in z score values tended to correlate with the time that elapsed after the cessation of treatment, but it was non-significant. The rate of osteoporosis (z score <-2 SD) was found 44.3% whereas 41.4% of children were found osteopenic (-1<z score<-2). Cumulative steroid dose in all risk groups and cranial radiotherapy had no effect on BMD. BMD was found low in passive smokers, but it was non-significant. There was a negative correlation between BMD and the duration of television watching (p<0.01). BMD and daily calcium intake showed positive correlation (p<0.001). Serum IGF-1 and 25(OH) D vitamin levels were significantly low in ALL patients than in control group (p<0.001). However, the levels in each leukemia risk group were not different (p>0.05). Serum IGF-1 had also positive correlation with BMD (p<0.01). Logistic regression analyses revealed that the most significant factor influencing BMD was daily calcium intake (OR: 0.997; 95%CI: 0.995-0.999). Conclusion. BMD was determined in 85% of children. Daily calcium intake was the most significantly factor influencing BMD. Serum IGF-1 levels was also found valuable in determining the severity of osteoporosis in leukemic children.
Chronic myeloid leukemia III

0672
CYTOTOGENIC AND MOLECULAR MONITORING OF MINIMAL RESIDUAL DISEASE IN PATIENTS WITH CML IN CHRONIC PHASE ON IMATINIB MESYLATE THERAPY: THE SINGAPORE GENERAL HOSPITAL EXPERIENCE

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Introduction. Imatinib mesylate (Glivec) has been demonstrated to induce good haematologic and cytogenetic response rates in patients with Philadelphia (Ph)-positive chronic myeloid leukaemia (CML). In addition the development of quantitative PCR technology has enhanced our ability to monitor response and minimal residual disease (MRD) at the molecular level. Ann. Over a 3-year period from 2002 to 2004, 45 patients in chronic phase CML were treated with Glivec 400 mg/day at our institution. Quantitative PCR (polymerase chain reaction) for p210 BCR-ABL transcripts was performed at regular intervals to determine molecular response. Bone marrow studies were also done to determine cytogenetic response. Methods. Real-Time quantitative PCR was performed on the ABI PRISM 7700 Sequence Detection System following the procedures established by the Europe Against Cancer Program. We defined major molecular response (MMR) as a 3-log reduction in BCR-ABL/ABL ratio from the median baseline of 152%, and complete molecular response (CMR) as BCR-ABL undetectable or a 4-log reduction in BCR-ABL/ABL ratio. Results. The median age of the patients was 45 years (range, 18-76 years) and median follow up was 31 months (range, 11-49 months). Thirty patients (67%) achieved complete cytogenetic remission (CCyR) at 12 months and on further follow-up, another 13 attained CCyR in 18-32 months (median, 24 months). Thus, a total of 43/45 (95.5%) patients were able to achieve CCyR. Among these 43 patients, 27 achieved MMR or CMR at a median of 24 months (range, 6-41 months). Twenty-three patients had subsequent PCR analyses and molecular response was sustained in 10 (43%) patients.

Table 1. Cytogenetic and molecular responses of patient cytogenetic and molecular responses of patients.

<table>
<thead>
<tr>
<th>Total</th>
<th>No who achieved MMR</th>
<th>No who achieved CMR</th>
<th>No who failed to achieve MMR</th>
<th>No who failed to achieve CCyR</th>
</tr>
</thead>
<tbody>
<tr>
<td>45</td>
<td>30</td>
<td>11</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>CCyR12</td>
<td>30</td>
<td>11</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>CMR12</td>
<td>30</td>
<td>11</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>No</td>
<td>No who achieved MMR</td>
<td>No who achieved CMR</td>
<td>No who failed to achieve MMR</td>
<td>No who failed to achieve CCyR</td>
</tr>
<tr>
<td>0-14 months</td>
<td>18</td>
<td>11</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>15-24 months</td>
<td>30</td>
<td>11</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>25-32 months</td>
<td>30</td>
<td>11</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>33-45 months</td>
<td>30</td>
<td>11</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>46-50 months</td>
<td>30</td>
<td>11</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>51-60 months</td>
<td>30</td>
<td>11</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>61-72 months</td>
<td>30</td>
<td>11</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>73-120 months</td>
<td>30</td>
<td>11</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>121-180 months</td>
<td>30</td>
<td>11</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>&gt;180 months</td>
<td>30</td>
<td>11</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

One patient lost her CCyR with 5% Ph-positive metaphases detected at 80 months and MMR achieved at 18 months was lost at 27 months. This correlated well with increasing BCR-ABL/ ABL ratios from 0.061% (18 months) to 1.071% (32 months). Of the 2 patients who did not achieve CCyR, one remained refractory with less than 0.5 log reduction in BCR-ABL transcript levels after 2.5 years on Glivec. This patient recently had an allogeneic BMT, achieved major cytogenetic response and a 1-log reduction 1 month later. Glivec was then restarted. Further monitoring of cytogenetic and molecular responses is necessary for this patient and the last patient whose latest evaluation was at 18 months.

Conclusions. Overall, 43/45 (95.5%) of patients achieved CCyR in a median of 12 months (range, 0-32 months). This incidence rate appears to be higher than those previously reported (75%-90%). MMR or CMR was achieved in 27 patients (60% of all patients). While CCyR is sustained in most patients, molecular response is sustained in only 43% of patients. We observed that patients with fluctuating levels around 2-2.5 log reduction, remained in CCyR while a trend of persistently rising BCR-ABL/ ABL transcripts could lead to a loss in CCyR. As data on molecular responses of Glivec-treated CML patients in Asia is limited, we would continue to accrue such patients for molecular monitoring to assess the association of molecular response with prolonged progression-free and overall survival.

0673
KINETICS OF TWO CO-EXISTING MUTATIONS IN THE BCR-ABL KINASE DOMAIN IN FOUR CML PATIENTS

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Background. Kinetics of mutant CML clones helps in better understanding their functional role. Aims. Investigation of kinetics of co-existing mutations in CML patients Methods and Results. Pyrosequencing was used to study the kinetics of the mutant Ph+ clones. BCR-ABL transcripts were quantified by Taqman real time PCR. Where applicable, RFLP studies were used for confirmation. Of the Ph-positive CML patients treated with imatinib (IM) at our institution who were screened for KD mutations as described previously, we were able to monitor the kinetics of the mutant clones in 4 patients, each of whom had two distinct KD mutations. Patient no. 1 failed to respond to IM and was found to have a F315I mutation. Year 255F treatment was changed to dasatinib, whereupon BCR-ABL transcript levels fell initially by >2 logs and the mutant clone became transiently undetectable. Thereafter transcript numbers increased and quantitative single nucleotide polymorphism (Q-SNP) using pyrosequencing and sequence analysis showed that the second Ph-positive clone consisted almost entirely of mutant cells with both Y255F and T315I mutations. Q-SNP analysis suggested the two mutations were both present in 95% of cells; this was confirmed by restriction enzyme digestion of polymerase chain reaction (PCR) product. Patient no. 2 showed a similar sequence of events. She responded poorly to IM and was found to have F311L mutant clone. She responded briefly to dasatinib with transient reduction in total BCR-ABL transcripts and disappearance of the mutant clone. Thereafter BCR-ABL transcript numbers increased rapidly; the F311L mutation reappeared but a T315I mutation was also detected at the same level, namely 92%, which suggests again that the two mutations co-existed in the same sub-clone (therefore probably in cis). In contrast, patient no. 3 had no significant reduction in total BCR-ABL transcript levels despite treatment with IM. Both Y255F and M351T were detected but Q-SNP data showed that they represented on average 10% and 60% respectively of the total transcript numbers, suggesting involvement of different Ph-positive sub-clones. Similarly, patient no. 4 achieved complete cytogenetic remission with IM; after treatment for 1 year 90% of transcripts had a M351T mutation and 10% a T315I mutation. After two years on IM he still had the M351T mutation (90%) but a new mutation, H396R, was detected in 46% of transcripts. Thereafter, however, the levels of the two mutations evolved discordantly; for example after 30 months on IM the M351T mutation comprised 45% of transcripts whereas the H396R comprised 20%. These values imply that the two mutations involved distinct sub-clones (therefore in trans) which had different degrees of sensitivity to imatinib. Conclusions. These observations provide further evidence for the sequential acquisition of mutant clones, which may differ in their responsiveness to specific tyrosine kinase inhibitors. It supports the notion that the best method of preventing resistance may be to start treatment with a combination of more than one tyrosine kinase inhibitor.

0674
PROGNOSTIC SIGNIFICANCE OF THE LEVEL OF RESIDUAL DISEASE AFTER 12 MONTHS IMATINIB BASED THERAPY: THE GERMAN CML-STUDY IV

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Background. Targeted therapy with imatinib induces high response rates in chronic myeloid leukemia (CML) patients (pts). However, about 4% of pts per year relapse with reappearance of Ph chromosome positive metaphases or loss of hematologic control. Aims. We sought to investigate the relationship between BCR-ABL transcript levels at month 12,
cytogenetic response and relapse free survival after 2 years of imatinib-based treatment within the German CML-Study IV. Methods. Between July 2002 and January 2006 731 pts were randomized of whom 251 pts were recruited until November 2003 and thereby qualified for a 2-year evaluation. In 189 pts quantitative RT-PCR data at month 12 after start of treatment are available. In parallel pts were monitored by conventional cytogenetics, qRT-PCR of the bcr/abl fusion gene transcript and mutation analysis of abl kinase domain. A classification of molecular response levels in 4 cohorts was applied: Ratios BCR-ABL/ABL of 0.01%, 0.12%, and 1.4% represent a 4, 3, and 2-log-reduction from a predefined baseline (IRIS definition), respectively. Results. After 12 mo of imatinib-based therapy, ratios <0.01% were achieved in 11 pts (cohort 1, 6%), ratios of 0.01-0.12% in 56 cases (cohort 2, 30%), >0.12-1.4% in 70 pts (cohort 3, 37%), and >1.4% in 52 pts (cohort 4, 27%). The 2-year assessment showed CCR in 7/7 evaluable pts in cohort 1 (100%), 29/29 evaluable pts in cohort 2 (100%), 33/34 evaluable pts (97%) in cohort 3, and 12/22 evaluable pts in cohort 4 (55%, p<0.0001). Ratios BCR-ABL/ABL after 2 years differed significantly between cohorts (cohort 1 0.011%, cohort 2 0.060%, cohort 3 0.39%, cohort 4 6.4%, p<0.0001). Two pts who achieved CCR at month 12 experienced cyto- genetic relapse (12 and 32% Ph+ metaphases) at month 24. Their 12 mo BCR-ABL/ABL ratios were 2.8% and 2.2%, respectively, in contrast to 0.11% which represents the median ratio of those pts achieving CCR at month 12 which was ongoing at least until month 24. Taken together pts lacking any mutations (n=15, median 52% Ph+ metaphases) revealed significantly higher BCR-ABL transcript levels after 12 months than those with CCR at 2 years (13.8% vs 1.7%, p<0.0001). Within 2 years of observation 16/251 pts (6%) progressed to blast crisis, of whom two revealed clonal evolution (complex aberrant karyotype, n=2), and another two developed BCR-ABL kinase domain mutations detectable by D-HPLC and conventional sequencing 11 mo (M244V) and 1 mo (E450G) before hematologic diagnosis of blast crisis. Conclu- sions. The assessment of BCR-ABL transcript levels by quantitative RT-PCR at month 12 of imatinib-based therapies shows prognostic significa- nce for 2-year cytogenetic and molecular response. Long term obser- vations will demonstrate its impact on prediction of long term response.

0675
MUTATION ANALYSIS OF THE KINASE DOMAIN OF THE BCR/ABL FUSION GENE IN CHRONIC MYELOGENOUS LEUKAEMIA
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Background. Imatinib induces complete cytogenetic remission in a high proportion of CML patients. However, patients in cytogenetic remis- sion usually display residual bcr-abl positive progenitors by RT-PCR. The mechanisms underlying persistence of small numbers of malignant progenitors in imatinib-sensitive patients are unclear. Aims. To gain more information about the biology of bone marrow metaphases. A classification of molecular response levels in 4 cohorts was applied: Ratios BCR-ABL/ABL of 0.01%, 0.12%, and 1.4% represent a 4, 3, and 2-log-reduction from a predefined baseline (IRIS definition), respectively. Results. After 12 mo of imatinib-based therapy, ratios <0.01% were achieved in 11 pts (cohort 1, 6%), ratios of 0.01-0.12% in 56 cases (cohort 2, 30%), >0.12-1.4% in 70 pts (cohort 3, 37%), and >1.4% in 52 pts (cohort 4, 27%). The 2-year assessment showed CCR in 7/7 evaluable pts in cohort 1 (100%), 29/29 evaluable pts in cohort 2 (100%), 33/34 evaluable pts (97%) in cohort 3, and 12/22 evaluable pts in cohort 4 (55%, p<0.0001). Ratios BCR-ABL/ABL after 2 years differed significantly between cohorts (cohort 1 0.011%, cohort 2 0.060%, cohort 3 0.39%, cohort 4 6.4%, p<0.0001). Two pts who achieved CCR at month 12 experienced cyto- genetic relapse (12 and 32% Ph+ metaphases) at month 24. Their 12 mo BCR-ABL/ABL ratios were 2.8% and 2.2%, respectively, in contrast to 0.11% which represents the median ratio of those pts achieving CCR at month 12 which was ongoing at least until month 24. Taken together pts lacking any mutations (n=15, median 52% Ph+ metaphases) revealed significantly higher BCR-ABL transcript levels after 12 months than those with CCR at 2 years (13.8% vs 1.7%, p<0.0001). Within 2 years of observation 16/251 pts (6%) progressed to blast crisis, of whom two revealed clonal evolution (complex aberrant karyotype, n=2), and another two developed BCR-ABL kinase domain mutations detectable by D-HPLC and conventional sequencing 11 mo (M244V) and 1 mo (E450G) before hematologic diagnosis of blast crisis. Conclu- sions. The assessment of BCR-ABL transcript levels by quantitative RT-PCR at month 12 of imatinib-based therapies shows prognostic significa- nce for 2-year cytogenetic and molecular response. Long term obser- vations will demonstrate its impact on prediction of long term response.

0676
BENEFIT OF IMATINIB AT 600 MG/DAY FOR PATIENTS WITH PHILADELPHIA CHROMOSOME POSITIVE CHRONIC MYELOGENOUS LEUKEMIA IN ACCELERATED PHASE: A RETROSPECTIVE STUDY OF 44 PATIENTS
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Background. Chronic myelogenous leukemia (CML) is a malignant hematologic disorder with a poor prognosis in advanced stages of the disease. Currently, the only curative approach is based on allogenic stem cell transplantation. Therefore, several studies have addressed the role of imatinib Mesylate (IM) in the treatment of CML. Studies have showed that CML patients treated with imatinib Mesylate (IM) have provided tremendous and significant improvement in chronic phase of the disease with cytogenetic remissions rates above 75% in numerous studies. The benefit and the dose of IM in accelerated phase remain uncertain. Some few studies have suggested that IM at 600 mg/d could increase the cytogenetic rate with a median survival close to 4 years. We report on a single and retrospective experience with IM 600 mg/d in 44 accelerated phase CML patients (pts). Patients and Methods. 44 adult pts (M = 27, F = 17) with accelerated phase CML (IBMTR criteria) have been treated with IM at 600 mg/d. 28 pts had received a previous treatment before IM. The median time between diagnosis and IM treatment is 13.85 months (0.005-189.42). We analyzed the cytogenet- ic and molecular responses, the overall survival and tried to determine factors possibly linked with survival. Statistical analysis have been per- formed with Kaplan-Meier method and the comparative curves with the log-rank method. Results. The median age at IM start is 51,18 years (24-80). The median follow-up time is 29,24 months. Sixteen pts died and 23 are still alive. 56 pts (84%) have a complete hematologic response, 27 (61%) a major cytogenetic response (MCyR) from whom 21 (78%) a complete response (CCyR). The probability to be in CCyR is 58% (± 17%) with a median time of 11,6 months (CI 95% : 00,0-32,7). 11 pts have a major molecular response (MMR) with a 3 log decrease of BCR-ABL levels in 8 pts reached a complete molecular response (CMR). The median survival is 46,89 months. A CCyR at 3 months, and a MMR seem to be determinant for the survival (respectively p<0.089 and p<0.0067) while anemia and a previous treatment appeared to affect negatively the survival (respectively p<0.0646 and p<0.029). Conclusion. This study emphasizes the benefit of IM at a dose of 600 mg/d in accel- erated phase CML pts with substantial cytogenetic and molecular responses. A CCyR at 3 months and a MMR seem to be determinant on the progressive free survival. These results should be taken in con- sideration in the management of such pts particularly when the question of allogeneic stem cell transplantation is raised.

0677
PRAME AS A SECONDARY TARGET FOR BCR-ABL-POSITIVE LEUKEMIAS
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Background. Chronic myeloid leukemia (CML) is a myeloproliferative hematologic disorder characterized by a clonal expansion of neoplastic hematopoietic stem cell. The neogene Bcr-Abl is a hallmark of CML and it is the
result of the fusion of bcr and c-abl genes. The correlation between the bcr-abl and p210BCR-ABL fusion protein is probably independent of the Bcr-Abl's tyrosine kinase activity. On the other hand, a higher expression of p210 was related to a disease progression, as we found 8-times more p210 in accelerated than in chronic phase and 29-times more in blastic than in chronic phase and no p210 expression was found in cytogenetic remission post-imatinib. Conclusions. Recently a function of bcr-abl was described as a dominant repressor of retinoic acid receptor (RAR) signaling. Signaling through RAR induces proliferation arrest, differentiation, and apoptosis in many cell types. Considering the function and our results, we can suggest that new therapeutic approaches can be developed, aiming to inhibit the function or expression of this gene, for the most delayed phase of the illness, in imatinib-refractory patients.

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0678 GENETIC CHARACTERIZATION OF 203 DE NOVO CHRONIC MYELOID LEUKEMIA PATIENTS IN THE PORTUGUESE POPULATION


Background. Philadelphia chromosome (Ph1) is the hallmark of almost all the cases of CML. The vast majority of patients express either the b2a2 (e1a2s) or b3a2 (e1a2) BCR-ABL mRNA, characteristic of the p210BCR-ABL fusion protein. A very few patients express the e1a2 mRNA, characteristic of the p190BCR-ABL fusion protein and present in half of the adults who have BCR-ABL positive Acute Lymphoblastic Leukemia (ALL). However, some patients have the protein p230BCR-ABL in which p210 expression was found in cytogenetic remission post-imatinib. Conclusions. We identified the type and frequency of BCR-ABL fusion transcripts and Ph1 chromosomes in 203 Portuguese patients with de novo CML. Clinical diagnosis was confirmed by cytogenetic and/or molecular biology studies. Methods. Karyotypes were performed according to standard procedures. Molecular analyses were performed according to the BIOMED-1 Protocol. 203 patients with de novo CML were studied (Table 1).

Table 1. Portuguese patients with the novo CML used in this study.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Average age</th>
<th>Male</th>
<th>Female</th>
<th>Karyotype</th>
<th>Molecular Biology</th>
</tr>
</thead>
<tbody>
<tr>
<td>203</td>
<td>55.5</td>
<td>100 (49.3%)</td>
<td>103 (50.2%)</td>
<td>131 (64.5%)</td>
<td>180 (88.7%)</td>
</tr>
</tbody>
</table>

Results. Ph1 chromosome was found in 96.2% of patients; 3.8% were Ph1 negative BCR-ABL positive. In the Ph1 positive group almost 6% had variants (t9;22;V), (t9;22) or (t9;V) and 12.2% had additional anomalies, while the remaining (77.9%) presented the standard karyotype [46,XX,t(9;22)(q34;q11)] or [46,XY;9(22)q34]. 767 of CML patients expressed only BCR-ABL, p210 transcripts; 2.2% co-expressed p210 and p190 transcripts; while 2.5% expressed BCR-ABL p190 (1.7%) or p230 (1.1%) e1a2a, each one with a frequency of 0.56%. Conclusions. Our cytogenetics findings do not significantly differ from those described by other authors, except for the frequency of the Ph1 negative BCR-ABL positive cases, which is slightly below the one reported. Based on molecular biology studies a discrepancy regarding BCR-ABL expression is shown. According to the literature more than 99% of patients express p210 transcripts, while the remaining express BCR-ABL p190 and other variants, considered rare. In our population the frequency of non BCR-ABL p210 transcripts is higher than the one reported (1.7% for patients expressing p190 and 1.1% for atypical transcripts).

Different transcripts may result from alternative splicing between BCR and ABL and within BCR itself. RNA splicing implies the recognition of consensus sequences, including 5’s and 3’s splice sites and a weakly conserved branchpoint in the intron upstream the 3’s splice site. Polymorphisms affecting these sequences could activate cryptic branchpoints that are less efficiently used originating unusual products. Being so, the frequency of atypical transcripts in our population might reflect a specific genetic background. Nevertheless, the complete characterization of BCR-ABL transcripts, namely the uncommon ones, will ascertain correlations with different disease phenotypes and improve the outcome of single patients by individualization of therapeutic strategies.

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0679 GENE EXPRESSION PROFILING IN CML PATIENTS RESISTANT TO TREATMENT SPECIFIC PROFILES IN NON-RESPONDERS WITH LOW BCR-ABL TRANSSCRIPT LEVELS

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CML is characterised by a presence of fusion gene bcr-abl. The level of bcr-abl transcript characterises the disease status and bcr-abl kinetics are an important prognostic factor. However, we found that among patient resistant to therapy there were those whose low bcr-abl levels did not correlate with the disease status. Moreover, patients with non-correlating bcr-abl levels had the worst clinical outcome. Our aim was to find gene expression differences underlying this discrepancy. To do this we turn to gene expression profiling using cDNA macroarrays. We analysed 28 samples of patients not responding to treatment. There were samples with bcr-abl levels corresponding (n=21) and not corresponding (n=7) with the clinical state of disease. Hierarchical clustering showed that out of 28 samples of non-responding CML patients all 7 samples with bcr-abl level not correlating with the disease status occupied a single cluster, clearly visible on the gene expression matrix. Among gene clusters our focus was kept on genes differentially expressed in non-correlating samples compared to the rest of the non-responders. We found clusters with genes up-regulated in non-correlating samples as well as clusters with genes down-regulated in these samples. Among up-regulated genes there were BAD, CDRN2A, O-6-alkylguan-DNA methyltransferase, Notch4, RhoC and VEGFR1. Clusters of down-regulated genes included e.g. Akt2, MAPK8, cyclins A, G1 and D3 and several caspases. In conclusion, we have found a group of CML patients not responding to the treatment whose BCR-ABL transcript levels were not correlating with the clinical disease status. This group was characterised to have clearly different gene expression profiles to the other non-responding patients. The genes differentially expressed in these samples are candidates for further investigations on mechanisms of both therapy resistance and possible lose of BCR-ABL dependency in CML. The BCR-ABL independency in these patients was further supported with our preliminary data on Western blot analyses and other kinase inhibitor experiments.

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0680 THE EXPRESSION OF PROTO-ONCOGENES IN THE COURSE OF CHRONIC MYELOID LEUKEMIA

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Background. The chronic phase (CP) of chronic myelogenous leukaemia (CML) is characterised by the presence of chimeric bcr-abl gene and a prolife rate growth of mature polymorphonuclears. The accelerated
phase and blast crisis (AP and BC) of CML may show additional oncogenic aberrations and pronounced anaplasia manifested by an increase in organomegaly and blast count. The abnormal expression of some proto-oncogenes which may accompany or even precede BC of CML warrants their study. Aim. The follow-up of oncogene expression during the course of CML. Methods. We studied 85 patients (pts.) with the median age of 50 (range 16-75 years). Among those, 30 had been treated with imatinib. The commencement of the study in these pts. were in CP, 25 in an AP, and 31 in the BC. The temporal expression (percentage positivity per 1000 analyses) of c-kit, c-myc, H-Ras, cyclin A1, p53, bcl-2 and VEGF proto-oncogene proteins over the course of CML was studied using the immunohistochemical technique which utilizes relevant monoclonal antibodies. It was correlated with the laboratory (Hb, WBC and platelet counts, and the percentage of blasts) and clinical parameters (organomegaly, duration of CP, AP, and BC) of disease progression. Results. The level of c-kit expression differed significantly in time with the largest values observed in the BC (x2, p=0.025). The level of anti-apoptotic protein bcl-2 increased significantly with the progression of CML (x2, p=0.005). Conversely, the expression of c-myc was highest in CP (x2, p=0.083). The expression of VEGF protein was most pronounced in an AP (ANOVA, p=0.033). There was no significant difference in the level of expression of H-Ras, cyclin A1 and p53 over the course of CML. The level of VEGF expression correlated inversely with degree of organomegaly (Pearson, r=-0.49, p=0.01). The c-kit expression correlated directly with the extent of marrow fibrosis (Spearman, r=0.407, p=0.000). High expression of VEGF correlated with a longer duration of CP (log rank, p=0.0304) and with a longer overall survival (log rank, p=0.042). Conclusion. The significance of changes in oncogene expression, estimated by a histochemical approach over the course of CML, should be taken into consideration of clinical importance in the treatment and prognosis of these patients. The details of the temporally-related changes in oncogene expression in leukemia cells require the study at the molecular level.

0681
P190 BCR-ABL CHRONIC MYELOID LEUKEMIA PARTLY RESEMBLING CHRONIC MYELOMONOCYTIC LEUKEMIA IN A YOUNG PATIENT TREATED WITH IMATINIB
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Background. In Ph-positive CML, the breakpoint on the BCR gene nearly always occurs within the major breakpoint cluster region (M-bcr), and the BCR-ABL fusion gene encodes a protein of 210 kDa molecular weight (P210). In most Ph-positive ALL, by contrast, breakpoints occur 22 occurs within the first intron of the BCR gene, or minor bcr (m-bcr). In these cases, the first BCR exon is fused to ABL exon 2 (e1a2 junction) and a BCR-ABL protein of 190 kDa is formed (P190). This form of CML was reported as having some unusual clinical and haematological features, partly resembling chronic myelomonocytic leukemia (Melo et al., 2000). The first P190 CML case reported in a very young patient, in contrast with other Ph-negative CML (median 53.5) . It remains to be seen whether the long term response to imatinib in this type of CML will compare to that observed in classical P210 cases or will resemble more the poorer response achieved in Ph-positive ALL.

0682
ANGIOGENIC ACTIVATORS IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA: EFFECT OF TREATMENT WITH IMATINIB MESYLATE
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Background. Angiogenesis is nowadays considered an important factor in the maintenance of vascular and extra-vascular chronic myeloid leukemia (CML). Several studies have recently reported elevated levels of angiogenic activators such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) in CML patients. However, there have been only few data on the influence of imatinib mesylate (IM) treatment on the levels of angiogenic cytokines in CML. Aims. To analyze peripheral blood levels of angiogenic activators in patients with newly diagnosed CML and during imatinib treatment. Methods. We measured plasma concentrations of VEGF, bFGF and soluble endoglin (sCD105) using sandwich enzyme-linked immunosorbent assay (ELISA) in 16 patients with chronic-phase CML and 80 healthy blood donors; furthermore, repeated samples during the therapy with (IM) were analyzed. Results. We found a statistically significant increase in VEGF (mean ± SD [standard deviation], 491.0 ± 365.5 vs. 64.2 ± 69.5 pg/ml, 95% CI [confidence interval] of mean, 296.4-685.7 vs. 510.7-77.5 pg/ml, p<0.0001) and sCD105 (mean ± SD, 7.0 ± 1.9 vs. 4.57 ± 1.51 pg/ml, 95% CI of mean, 5.8-8.18 vs. 4.20-4.93 ng/ml, p<0.0001) but not bFGF (p=0.060) in comparison to the control group. VEGF levels significantly decreased in 7 patients who achieved hematological remission (6 complete remissions, 1 partial remission) during therapy with IM (mean ± SD, 679.6 ± 431.5 vs. 152.7 ± 63.3 pg/ml, 95% CI, 280.6-1076.6 vs. 74.1-191.5 pg/ml, p=0.015). There was no significant change in bFGF or sCD105 (p=0.856 and 0.125, respectively). Conclusions. We found significantly elevated VEGF and sCD105 levels in CML patients. In addition, successful treatment with IM resulted in significant decrease of VEGF. These data lend further support to the importance of angiogenesis in pathophysiology of CML. Further studies incorporating larger number of patients are needed to confirm our findings. Supported by research project MZO 00179906 from Ministry of Health of Czech Republic.

0683
THE E19A2 BCR-ABL BREAKPOINT: MORE FREQUENT THAN OTHER ATYPICAL BCR-ABL VARIANTS IN CHRONIC MYELOGENOUS LEUKEMIA?
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In the vast majority of patients diagnosed as having chronic myelogenous leukaemia (CML) and t(9;22), the breakpoint on chromosome 22 occurs in the major region of the BCR gene (M-BCR); this translocation usually results in a hybrid BCR-ABL mRNA with a b2a2 and/or b3a2 junction, which encodes a p210 fusion protein proved to be involved in the mechanism that underlines the chronic phase of CML. Here, we report 7 newly diagnosed chronic phase CML patients with an unusual e19a2 BCR-ABL transcript. The BCR breakpoint in this type of rearrangement occurs downstream from M-BCR, in the m-BCR region, between exons e19 (c5) and e20 (c4). This novel translocation, previously reported in our group in only few patients, results in the transcription of e19a2 type BCR-ABL fusion mRNA, which is translated into a p230-kD BCR-ABL protein. We observed that in some patients e19a2 was associated with neutrophilic leukaemia while in the other patients the rare rearrangement was associated with a classical CML in chronic phase. In particular, in a 45-year-old male hemoglobin was 14.7 g/L, white blood cell count 7.1 ×10^11/L, neutrophils 64%, lymphocytes 8%, monocytes 2%, eosinophils 3%, basophils 5%, neutrophils 7%, lymphocytes 9%, eosinophils 2% and platelet count 277 ×10^11/L. In a 30-year-old female hemoglobin was 9.4 g/L, white blood cell count 108 ×10^9/L, neutrophils 43%, lymphocytes 4.8%, monocytes 4.3%, eosinophils 0.9%, basophils 2%, promyelocytes 2% and platelet count 277 ×10^11/L. In all 7 patients cytogenetic analysis of 20 bone marrow
metaphases, using G-bandning, showed the t(9;22)(q34;q11) in all cells. BCR-ABL was detected by multiplex PCR analysis of chromosome banding (Giemsa), using four primers to generate PCR products from BCR-ABL and normal BCR gene transcripts. This resulted in a band of about 900 bp, in addition to the same 808-bp band representing the BCR transcript. Using two of the multiplex primers (B2B, 5’ACAGATTTGCCGCTACCAT- CAAATAAG 3’; and C3A, 5’TGTTGACTGCGTGATGTAGTGCCT TGG 3’), the BCR transcript was generated, indicating that no additional sequence was due to exons downstream of e14(b1). Sequencing of the PCR products after amplification with specifically designed primers revealed an in-frame BCR-ABL e19a2 transcript. It is interesting to note that in one of 7 patients there were both a b3a2 and e19a2 transcripts. We conclude that BCR-ABL transcript is not so rare transcript in newly diagnosed CML patients.

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0684  
GENE EXPRESSION PROFILE OF PATIENTS INNATELY RESISTANT TO IMATINIB MESYLATE  
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Background. Imatinib (IM) a specific ABL tyrosine kinase inhibitor has been reported to have a significant clinical effect on chronic myeloid leukemia (CML). Some patients treated with IM acquire resistance probably due to selective pressure on cells that carry amplified copies of of the BCR-ABL oncogene or point mutations in the ABL affecting the binding site of the drug. In other cases resistance appears to exist prior to drug exposure. Such innate mechanism of resistance is poorly understood, some evidences suggest that activation of alternative pathway, may confer BCR-ABL independent survival to CML cells. Comparative genome expression studies have long been known to provide important insight into biological process such as proliferation, differentiation, apoptosis and transformation. Only few gene expression profiling-based studies of CML and IM treatment have so far published. Moreover, only three studies has been performed on patient’s samples, resulting on heterogeneous conclusions. Aims. To investigate about the molecular events involved in innate IM resistance in CML we compared the expression profile of a set of 380 genes on resistant patients versus responder patients. We chosen 380 genes involved in process like apoptosis, cell adhesion, cell proliferation, signal transduction, chromosome/DNA dynamics. Methods. A set of 15 patients (3 female, 10 male, median age 50) with CML was selected from several diagnosed at Division of Hematology, Polyclinic Hospital of Palermo. Patients were defined as responder to IM if they achieved reduction of BCR/ABL transcript greater than 3 log within 6 months, while resistant those with less than 1 log of reduction after 6-12 months of treatment. We use the TaqMan MicroFluidic Card (Applied). This technology is a method for real-time RT-PCR that can simultaneously assay the RNA expression levels of up to 380 genes on a single card. RT-PCR data were quantified using the SDS 2.1 software and normalized using the GAPDH as endogenous control. Results. After the analysis of seven responder and six no responder samples we detected differential expression of 18 genes that correlate with the imatinib resistant phenotype. The resistant cells over express (1.9 fold /7.5 increase) genes of different categories: signal transduction (SO51,PEA151STAT5B), apoptosis (BCL2, BAX), genes involved in cell adhesion (SELL, ITGB7), genes related to cell cycle progression (CCND2, CDK4) and transcription factors genes (ETS2, SMAD1, KLIP). Conclusions. In the pathogenesis of CML the expression of BCR-ABL activates adhesion (SELL, ITGB7), genes related to cell cycle progression (CCND2, CDK4) and transcription factors genes that expression of BCR-ABL is not so rare transcript in newly diagnosed CML patients.

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0685  
PROGNOSTIC SIGNIFICANCE OF WT1 GENE EXPRESSION IN CHRONIC MYELOGENOUS LEUKEMIA TREATED WITH IMATINIB  
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Background. Despite the availability of imatinib (Glivec®, Novartis, NJ, USA) as the accepted standard approach to the treatment of newly diagnosed patients with CML, the overall management of the disease has become more complex than ever. The cytogenetic and molecular response to imatinib in patients in first chronic phase overrides the current major criteria for risk stratification, i.e. Sokal score. New baseline biological markers predicting the response to imatinib therapy would thus be valuable to identify patients in whom imatinib will fail, so that timely adjustments can be made to the overall therapeutic strategy. Aims. The objective of this study was to evaluate if the WT1 (Wilms tumor gene 1) gene expression at diagnosis bears any prognostic information, i.e. if it relates to the cytogenetic response obtained during the first year of imatinib therapy in chronic phase CML patients. Methods. Peripheral blood (PB) and bone marrow (BM) samples were obtained in 25 newly diagnosed chronic phase CML patients, before commencing imatinib treatment, for analysis of cytogenetic response. In addition, BM and PB were sampled at 3-month intervals to determine the cytogenetic and molecular response, respectively. Quantitative RT-PCR techniques (qRT-PCR) were used to measure BCR-ABL and WT1 transcript levels, and conventional karyotyping was used for measuring the cytogenetic response. Good cytogenetic responders were defined as patients having obtained a major cytogenetic response (MCGR), i.e. < 5% Ph-positive metaphases, within 1 year of treatment. Results. At diagnosis the seven CML patients with a suboptimal cytogenetic response were found to have significantly higher WT1 transcript levels compared to those 18 patients with a good cytogenetic response (MCGR) (p<0.02). This difference was seen both when peripheral blood and bone marrow samples were used as templates. No relationship was seen between BCR-ABL transcript levels at diagnosis and the cytogenetic response to imatinib therapy obtained within the first year of treatment. Conclusion. A high WT1 gene expression level at diagnosis might identify those CML patients that will have a suboptimal cytogenetic response to imatinib therapy. It appears warranted to study this hypothesis in a larger patient material.

0686  
MONITORING OF MOLECULAR RESPONSE TO STI OR STI-INFα OF CHRONIC MYELOID LEUKEMIA (CML) PATIENTS  

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Background. Despite the continuously increase control of CML obtained in the last two decades with IFNα-based regimens or STI used alone or in combination with other active agents, minimal residual disease (MRD) remains detectable in the majority of patients in complete karyotypic response to this non-transplantation therapeutic strategies. Aims. Evaluation of the molecular response rate to STI or to STI-INFα combination and the pattern of this type of remission over time. Methods. Thirty six Ph' positive CML patients (pts) were treated with STI at the time of this analysis. A molecular response was assessed by RT-PCR (van Dijck et al. Leukemia 2003 17;2318-57) or by quantitative reverse transcriptase PCR (RQ-PCR) (Gabert J et al. Leukemia 2003 17;23:819-57). Results. Molecular response was assessed in 13/16, 19/20, 3/5 and 5/11 pts of G1, G2, G3, G4, respectively. Ten pts were not evaluable because of STI (2) or INFα (3) discontinuation, patient refusal to perform the test (1) or too short treatment period (4) at the time of this analysis. A molecular response (RT-PCR) was documented at least once in 18 patients. In particular, it was documented in 7/13, 4/19 and 2/3 pts treated respectively with early or late STI or early STI-INFα combination. In 7 (3 in

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remission as defined by the absence of any detectable BCR/ABL transcript at March 2006. However, in spite of a rapid decline in BCR/ABL positive cells during treatment with 131-I, the patient did not achieve molecular remission at the moment of clonal detection as well as at the moment of this study. Clinical and cytogenetic data are summarized in Table 1. Conclusions. Complete molecular remission as defined by the absence of any detectable BCR/ABL transcript is usually observed only in patients who underwent a allogeneic bone marrow transplantation and is uncommon in imatinib treated cases. However, the data reported here indicate that a small proportion of CML patients could achieve eradication of the disease with standard imatinib treatment. It remains to be established if the previous therapy with interferon or other immunological mechanisms may contribute to this phenomenon.

### 0688

**CHRONIC MYELOID LEUKEMIA AFTER TREATMENT WITH 131-I FOR THYROID NEOPLASMS: TWO NEW CASES AND REVIEW OF THE LITERATURE**


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Background. Chronic myeloid leukaemia (CML) is a clonal disorder arising from a somatically mutated pluripotent stem cell. It is a process of unknown aetiology, although there is a higher incidence between populations exposed to irradiation: Japanese atomic bomb survivors, women treated with chemotherapeutic agents irradiated forankylosing spondylitis, among others. CML after treatment with 131-I for thyroid carcinoma is a rare condition. We report two new cases of CML associated with 131-I treatment for thyroid carcinoma, and a review of the literature. Case Report 1. A 48-year-old female was diagnosed in February 1993 of chronic lymphocytic leukaemia (CLL). She had a 2-year history of euthyroid multinodal goiter, and two years later she developed a papillary thyroid carcinoma. The thyroid gland was partially removed, and the patient began radiation therapy receiving a cumulative 131-I dose of 525 mCi, for ablation of thyroid remnants. Nine years later she developed leukocytosis and thrombocytosis, and Philadelphia (Ph) chromosome-positive CML was diagnosed. When she was on Imatinib treatment, and at the present time she has only a clonal lymphocytosis in blood and bone marrow. This case is a rare combination of CLL and CML 131-I-related, and it is possible the unique known to the literature. Case Report 2. A 41-year-old man presented in November 1999 with a history of short stature, weight loss, leukocytosis, and hepatosplenomegaly. Eight years before he was diagnosed of papillary thyroid carcinoma, and received radiotherapy with 131-I, total dose 127 mCi. Typical Ph-positive CML was diagnosed. Currently, the patient is in complete remission after treatment with Imatinib. The leukemic proliferation of CML is related with prior X-irradiation exposure of the haematopoitic stem cell. It appears to exclude the only bone marrow proven source for the induction of the BCR-ABL fusion gene by X-irradiation in vitro. Those cells bearing this gene are positively selected by virtue of a growth advantage in vivo. There is a delay of several years between the initial mutational event and the development of clinical symptoms that lead to the diagnosis of CML. It was calculated that the elapsed time from occurrence of a single cell containing the Ph chromosome to a leukemic burden of 100,000 cells/μL was 6.5 years. A literature review disclosed only 10 cases similar to ours. The earliest cases of CML were diagnosed 4 to 5 years after the exposition to radiation. Although there is no evidence to prove whether the development of CML after thyroid carcinoma represents a treatment-induced complication for patients treated with 131-I, it may need adiagnose bone marrow cell follow-up to investigate the appearance of myeloproliferative disorders such as CML.

### 0689

**FOLLOW-UP OF CML PATIENTS WITH CLONAL CYTOGENETIC ABNORMALITIES IN PH NEGATIVE CELLS DURING TREATMENT WITH IMATINIB**

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Background. Clonal cytogenetic abnormalities in Philadelphia (Ph) negative cells of patients with Chronic Myeloid Leukaemia (CML) treated with imatinib have been reported in the past years. The aberrations most frequently described are trisomy 8, monosomy 7 and deletion 20q. Few data are available regarding the outcome of patients treated with imatinib and with Ph negative cells during treatment with imatinib. Data regarding bone marrow findings and clinical outcome are presented. Methods. The three patients were studied by conventional cytogenetics (CC) and by FISH with the following probes: CEP8, 5q31 (EGR-1), 7q31 (D7S486), 20q12 (D20S108) and Vysis. All the patients had received imatinib for at least 9 months when the abnormal clone was first detected. Bone marrow was examined for morphologic dysplasia at the moment of clonal detection as well as at the moment of this study. Clinical and cytogenetic data are summarized in Table 1. Results. Patient 1 had myelodysplasia when the abnormal clone was first detected as well as at present. During a 26 months follow up, imatinib dossi had to be decreased because of progressive cytopenias resulting in the loss of complete cytogenetic response (CCR). Patient 2 also had bone marrow dysplasia when cytogenetic anomalies were found. CCR only lasted six months. At present, she is
in blastic phase 29 months later despite of imatinib, although never hav- ing received more than 300mg because of cytopenias. Patient 3 showed no morphologic signs of dysplasia at any moment. During a 24 months follow up he also required decreases in imatinib dose because of cytopenias. Conclusions. 1. Abnormal clones were always detected in Ph negative cells. 2. The three studied patients presented cytopenias that limit- ed increases on imatinib dose, independently of bone marrow morpho- logical features. 3. The correct control of the disease in these patients might be difficult because suboptimal doses of imatinib certainly con- tribute to treatment failure.

### Table 1. Clinical and cytogenetic data of patients when abnormal Ph negative clone was detected and at the moment of this study.

<table>
<thead>
<tr>
<th>Case</th>
<th>Gender</th>
<th>Age</th>
<th>Chromosome type (first analysis)</th>
<th>FISH at present</th>
<th>Chromosome type (at present)</th>
<th>Months of follow-up</th>
<th>Comments</th>
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**Non-Hodgkin’s Lymphoma - Clinical III**

**0690**

**THE OUTCOME OF 71 PATIENTS WITH PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA: A SINGLE INSTITUTION EXPERIENCE**

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Introduction. Primary mediastinal (thymic) large b-cell lymphoma (PMLBCL) is recognized as a separate subtype of diffuse large b-cell lymphoma with unique clinical and immunopathologic characteristics. The aim of our study was to evaluate clinicopathologic features and outcome of patients with PMLBCL. Methods: Between 1992 and 2005, 71 (66%:SM) previously untreated patients (pts) with PMLBCL were diagnosed and treated at our center. The median age was 56 years (range 16-66), 50 (70%) pts presented with CS IE-IIE, 58 (82%) had a superior venous cava syndrome, 25 (35%) pts presented with B symptoms, LDG was increased in 55 (77%), 18 pts (25%) had IFI score 3-5. The most frequent extranodal site was lung (35%), 51% pts had pleural effusion and 25% - pericardial effusion. 52 pts (82%) received CHOP as a first-line treatment, whereas other 19 pts were treated with other regimens (9 RCHOP and 10 MACOP-B). Mediastinal radiation therapy (RT) at dose 30-36 Gy was given to 24 (34%) pts. Results: Among 52 pts who received CHOP, 13 (25%) achieved a complete response (CR), 11 (21%) partial response (PR), 7 (14%) responding pts had early relapse. All pts, who relapsed/failed the initial treatment, underwent to salvage CT, 10 not responding pts had pro- gressive disease and died. Projected 3-years RFS and OS were 37% and 52% respectively. RCHOP and MACOP-B were highly effective regi- mens in all 19 pts (11 CR and 9 PR), but short follow-up and few num- ber of pts were not indicative for the superiority of this regimens over the CHOP. Conclusions. Our data confirm that most patients with PMBL- CBL had unfavorable chances for long-term survival when treated with CHOP. While R-CHOP or MACOP-B should now be the induction treat- ment of choice in the majority of PMLBCL cases. A longer follow-up is needed to finally define value of these regimens.

**0691**

**DIAGNOSTIC SENSITIVITY OF PET/CT IN PATIENTS WITH EXTRANODAL MARGINAL ZONE LYMPHOMA**

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Background. 18Fluoro-2-deoxyglucose (18F-FDG) positron emission tomography (PET) is a functional imaging technique currently widely used for initial staging, follow-up and monitoring response to therapy in patients with malignant lymphoma. However, its diagnostic accura- cy is overtly affected by the basic histopathology of the lymphoma sub- type. Extranodal marginal zone (MALT) lymphoma comprises about 8% of all NHL cases and is the third most common type of NHL. There is a controversy in the reported literature regarding the accuracy of 18F -FDG avidity in MALT lymphomas. While earlier studies suggested a limited role for PET in MALT patients due to their non-FDG avidity, a more recent report has suggested that this is incorrect. Recently, the technique of PET imaging was upgraded by using hybrid systems com- posed of PET and CT in the same framework. The fused functional-anatomic data appears to provide better localization of lesions, differ- entiating physiologic from malignant uptake and detection of unexpect- ed lesions otherwise overlooked. As yet no study investigating the role of PET/CT in MALT lymphoma has been reported. We hypothesized that since MALT may originate in organs with physiologic FDG uptake such as the gastrointestinal tract, the use of PET/CT may improve the diagnostic accuracy of 18F-FDG assessment. Aims. To evaluate the diag- nostic sensitivity of PET/CT in patients with MALT lymphoma and to assess its reliability in staging and monitoring disease activity. Methods. Thirty patients with biopsy proven MALT lymphoma in 33 sites, who underwent PET/CT at diagnosis, were included. Medical records, PET/CT findings and data obtained by other diagnostic procedures including gastroscopy were reviewed. Results. Common sites of MALT were the stomach (17), orbit (4), lung (2) and parotid (2). PET/CT detect-
ed active disease in 16 of 30 patients (53%). Sensitivity in gastric MALT (7/17, 41.1%) was lower compared to non-gastric MALT (9/15, 69%). The CT findings obtained from the fused PET/CT data allowed differentiation between physiological and pathological FDG uptake, especially in the stomach. PET/CT detected active disease in 7/7 (100%) patients with advanced disease (stage III-IV) but was positive in only 9/28 (32.1%) with early stage disease (I-II). The incidence of gastric FDG uptake was higher in patients presenting with gastric ulcer than in subjects with minimal or no macroscopic findings on gastroscopy. Of the 30 patients in the study cohort, nine had a follow up PET/CT after therapy. Of these, five biopsy proven relapse during follow-up. PET/CT detected relapse in 3 patients (including one patient who had negative PET on diagnosis). Conclusions: We report the initial results of a PET/CT imaging in MALT lymphoma patients. Our data suggest that PET/CT is a useful tool for both initial staging of disease and for follow-up after therapy. The anatomic data obtained from the CT part of the study allows for better interpretation of the corresponding scintigraphic abnormality detected on PET, mainly since MALT appears to involve organs which may be associated with physiologic 18F-FDG uptake.

**0692**

**BENIGN STRICTURES FOLLOWING TREATMENT FOR PRIMARY GASTRO-INTESTINAL NON-HODGKINS LYMPHOMA: CASE SERIES**

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**Background.** We report a novel complication, benign intestinal strictures, developing after treatment for gastrointestinal lymphoma (GINHL). Between 25 and 35% of non-Hodgkin’s lymphomas arise at extranodal sites, and around half of all extranodal lymphoma is in the gastrointestinal tract, the stomach being the commonest site. Treatment for GINHL carries a risk of internal haemorrhage, and perforation of an abdominal viscus, although studies have found that these complications are relatively rare, with incidence rates of around 0-2%. Methods. This is a retrospective case series. Five patients in whom a stricture complicated therapy for lymphoma were identified from the centre records over a six year period. Information was gathered from clinical records, radiology and stored histology samples. Available histology was reviewed by an independent histopathologist and x rays were reviewed by an independent radiologist.

![Figure 1. Duodenal stricture with replaced muscularis propria.](image)

Results. Three patients were male and two female. The median age was 55 (range 34-75). Three patients had localised diffuse large B-cell lymphoma of the small intestine (all in the duodenum or jejunum). One patient had post-transplant lymphoproliferative disorder affecting the small bowel and another follicular lymphoma involving the gastro-duodenal junction. In 3 cases resection due to bowel obstruction and in the other 2 this occurred during remission at 10 months and two years from the end of therapy, respectively. One case was treated with CHOP (cyclophosphamide, doxorubicin, vincristine and prednisolone) (having previously received rituximab at presenta-

**0693**

**ACTIVITY OF RITUXIMAB PLUS CYCLOPHOSPHAMIDE, DOXORUBICIN/MITOXANTRONE, VINCristine and Predesone (R-CHOP/R-CNOP) IN PATIENTS WITH RELAPSED MALT-LYMPHOMA**

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**Background.** Various chemotherapeutic agents as well as the anti-CD20 antibody rituximab (R) have been tested in patients with MALT lymphoma, but no standard chemotherapeutic regimen has emerged so far. Judging from data obtained in various types of lymphoma, the activity of R appears to be enhanced by combination with chemotherapy. As no data on this topic exist in MALT-lymphoma so far, we have analysed our experience with R plus cyclophosphamide, doxorubicin/mitoxantrone, vincristine and prednisone (R-CHOP/R-CNOP) in patients with relapsed MALT lymphoma. Patients and Methods. A total of 26 patients were treated; 15 were administered R-CHOP while 11 patients were given R-CNOP due to age >65 years or pre-existing cardiac conditions. In total, 13 patients presented in first relapse, 10 in second relapse, while the remaining 3 patients were given R-CHOP/CNOP in third relapse. A total of 16 patients (61%) had received at least one chemotherapeutic regimen before application of R-CHOP/R-CNOP, three had been pretreated with R, while various types of treatment including radiation, HP-eradication and surgery had been used for treatment before R-CHOP/CNOP. Cycles were repeated every 21 days, and restaging was performed after four cycles of therapy. In case of complete remission (CR), two further cycles were administered for consolidation while patients achieving partial remission (PR) or stable disease (SD) after restaging were given four further courses. Results. A total of 170 cycles were administered in our patients (median 6, range 2-8). Five of 26 cases (19%) had plasmacytic differentiation before initiation of therapy. Genetic aberrations specific for MALT-lymphoma were detected in 14 patients (54%). A high response rate was seen in our patients, as 19/26 (73%) achieved a CR and 7 patients (23%) had a PR, for an overall response rate of 100%. Response to treatment was not influenced by prior therapy, genetic aberrations or plasmacytic differentiation. Side effects were mainly haematological, with 8/26 patients (31%) developing leukocytopenia/granulocytopenia WHO grade III/IV. This was complicated by infection in a total of three patients (leve without a detectable infection in 2 and pneumonia requiring hospitalization in one patient). All eight patients were given prophylactic G-CSF for the following cycles, resulting in application of further chemotherapy as planned. Two patients developed thrombocytopenia WHO grade III. After a median follow-up of 19 months (range, 12-45), all patients are alive: 22 in ongoing remission, while 4 have relapsed 12-19 months after treatment. All relapses were histologically verified, and two of these patients showed plasmacytic differentiation upon relapse which had not been present before initiation of therapy with R-CHOP. Conclusions. This is the first analysis of a combination regimen including R in the treatment of patients with MALT lymphoma. Taken together, our data demonstrate that R-CHOP/CNOP is highly active and safe even in heavily pretreated patients with MALT lymphoma.
Background. The development of a Non-Hodgkin's lymphoma (NHL) is one of the most serious complications in patients with autoimmune diseases. Mucosa associated lymphoid tissue (MALT) lymphoma and diffuse large B-cell lymphoma (DLBCL) are the most common subtypes in these patients and chemotherapy is the therapy of choice in this setting. The combination of cyclophosphamide, doxorubicin, vincristine, prednisolone and rituximab (R-CHOP) seems to be the most effective regimen for lymphoma cell eradication at the moment. On the other hand, B lymphocytes are not only the key target in NHL but play also an integral part in the pathogenesis of autoimmunity. In keeping with these findings, one might hypothesize that immuno-chemotherapy administered to treat lymphoma might also diminish (or even eradicate) auto-reactive cell clones and might therefore improve the underlying autoimmune condition. Aims. As patients with B-cell lymphomas suffering from an underlying autoimmune condition undergoing therapy with R-CHOP offer the unique possibility of monitoring effects of therapy on various rheumatologic parameters, we have evaluated serologic autoimmunity markers and the clinical outcome of patients with autoimmune-conditions who received lymphoma treatment with R-CHOP during the course of their disease. Patients and methods. We have retrospectively analysed 15 patients with Non-Hodgkin's lymphoma who concurrently suffered from autoimmune diseases and were treated with the R-CHOP regimen (Table 1).

### Table 1. Patient characteristics.

<table>
<thead>
<tr>
<th>No.</th>
<th>Sex</th>
<th>Age</th>
<th>Lymphoma</th>
<th>Rheumatic Disease</th>
<th>RF</th>
<th>ANA C3c</th>
<th>C4</th>
<th>RD D0</th>
<th>Duration prior to NHL</th>
<th>Mediation prior to NHL</th>
<th>Immunomodulatory medication prior to NHL</th>
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<td>CSA</td>
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<td>Steroids, MTX</td>
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</tbody>
</table>


At every visit, patients were asked for the presence of joint pain, intake of corticosteroids, disease modifying anti-rheumatic drugs (DMARDs), non-steroidal anti-inflammatory drugs (NSAIDs) as well as quality of life. The rheumatoid factor (RF), antinuclear antibodies (ANA) and the complement factors C3c and C4 were measured immediately before institution of chemotherapy and then at regular intervals during the course of chemotherapy. Lymphoma response to treatment was classified according to the International Working Group recommendations. Results. The median levels of RF were 256 IU/ml (IQR: 12-960) before and 65.5 IU/ml (IQR: 20.25-577.75) after therapy (p=0.046). The median levels of ANA were 240 (IQR: 70-1600) before and 40 (40-160) after therapy (p=0.069). 10 (77%) patients showed clinical improvement of their autoimmune symptoms during the course of chemotherapy. Two (15%) patients reported no difference with regard to their autoimmune disease and one (7%) patient who suffered from rheumatoid arthritis experienced a worsening of her symptoms during therapy with R-CHOP. The autoimmune related symptoms recurred after a median time of 7 weeks (IQR: 6-8) in 7 patients. One patient who had suffered from vasculitis before initiation of had a durable remission after completion of R-CHOP now ongoing for 8 months. In terms of lymphoma-response, 11 patients achieved a complete- and 2 a partial remission. Summary/Conclusions. This analysis suggests that R-CHOP given for lymphoma treatment is also effective for therapy of concurrent rheumatic diseases. Both rheumatoid parameters as well as clinical symptoms showed a significant decrease during treatment with this immuno-chemotherapy. However, patients suffering from rheumatic diseases are at risk of developing lymphoma and should therefore be at least monitored for the duration of their lymphoma treatment. With regard to the lymphoma, R-CHOP displayed an excellent efficacy which seems comparable, or even better, to patients with NHL without autoimmune diseases.
Efficacy and Safety of DepoCyte (Liposomal Cytarabine) in Patients with Central Nervous System Involvement from Non-Hodgkin’s Lymphoma: A Report on 23 Patients Treated in Spain


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Background. Lymphomatous meningitis (LM) occurs in ~5% of patients with diffuse large B-cell lymphoma (DLBCL), and more frequently in patients with Burkitt’s lymphoma (BL) and lymphoblastic lymphoma (LL). Involvement of the CNS is demonstrated by multiple (2-3) intrathecal injections of cytarabine or methotrexate, which increases the burden on patients, carers and medical providers. As very few long-term survivors have been reported in any patient series, the major goals of therapy are relief from neurological symptoms, prevention of neurological progression, and preservation of quality of life. DepoCyte® is a sustained-release formulation of cytarabine for intrathecal injection, which maintains therapeutic concentrations in the cerebrospinal fluid (CSF) for 2 weeks. Treatment with DepoCyte® does not require an Ommaya reservoir, and offers the advantage of less frequent injections and potentially greater efficacy than conventional treatment. Methods. We report here on a series of 23 patients (median age 45 years, range 21-74; 13 male) with LM (9 relapsed) from 17 Spanish hospitals who were treated with intrathecal DepoCyte® (mean 3.5 injections, range 1-9) from March 2004 to December 2005. Concurrent dexamethasone was given as prophylaxis to brain metastasis. Results. Cytological responses (clearance of lymphoma cells from the CSF) were seen in 8 of 12 patients with DLBCL; 3 of 3 with BL; 1 of 1 with LL; 1 of 2 with mucosa-associated lymphoid tissue (MALT) lymphoma; 2 of 2 with follicular lymphoma; 1 of 1 with primary central nervous system lymphoma (PCNSL); and 1 of 2 with T-cell non-Hodgkin’s lymphoma (NHL). Neurological responses were seen in 8 of 12 patients with DLBCL (6 complete remissions (CR), 2 stable disease (SD)); 2 of 3 with BL (1 CR, 1 partial remission (PR)); 1 of 1 with LL (PR); 1 of 2 with MALT lymphoma (CR); 2 of 2 with FL (2 CR); 1 of 1 with PCNSL (SD); and 2 of 2 with T-cell NHL (1 PR, 1 SD). The overall response rate was 74% for both cytological response and neurological response (45.5% CR, 13% PR, 17.5% SD). Neurological progression occurred in 58% of patients, including 9 of 12 patients with DLBCL, after 7-830 months (2 alive at last report); 3 of 3 with BL after 28-90 months (2 alive); 1 with LL after 30 months (died); 2 of 2 with MALT NHL after 8 and 95 months (died); 2 of 2 with FL after 150 and 300 months (both alive); 1 of 1 with PCNSL (died); and 1 of 2 with T-cell NHL at 20 months (alive); 48% of patients remained alive at the last report. DepoCyte® showed good tolerability. Fifty-two per cent (12/23) of patients experienced no side effects, and the most common side effects associated with DepoCyte® injection were headache (n = 8), vomiting (n = 4) and nausea (n = 5), with one occurrence each of fever and neurological deficits. Conclusions. This series demonstrates the feasibility, safety, and tolerability of DepoCyte® in the treatment of LM associated with different histological subtypes of NHL. A substantial number of patients had a cytological response (74%) or a neurological response (74%), and 48% of patients were still alive at last report. Two-weekly (or monthly) DepoCyte® injections are much more convenient than the conventional alternatives. We consider that DepoCyte® may be the agent of choice for LM.

Lack of Humoral Response to Acute EBV Infection May Identify Patients with Fulminant EBV-Associated NK/T-Cell Lymphoproliferative Disorder


Singapore General Hospital, Singapore

Background. EBV associated NK/T-cell lymphoproliferative disorder is a rare and distinct clinical entity. In most instances, it is refractory to conventional treatment and confers a poor prognosis. We report the clinicopathologic features of 7 patients with EBV-associated NK/T-cell lymphoproliferative disorder treated between 2002 and 2005 in a single institution. Aims. We compare the presenting features and treatment outcomes of these patients. In doing so, we hope to identify trends that may help improve the diagnosis and management of this rare and fatal disease. Methods. The investigation is a retrospective study. Patients with the diagnosis of interest were identified from our lymphoma registry and clinical information of the cases obtained from pathological reports and clinicians. Results. All patients were of Asian origins (6 Chinese and 1 Malay). Other than a patient aged 66 years old with a history of renal transplant and was on immunosuppression, the other 6 patients were aged between 32 to 40 years with no significant past medical history. All patients had a preceding history of acute upper respiratory tract infection prior to their dramatic presentation. They demonstrated the haemophagocytic syndrome with severe systemic symptoms including high fever, acute pancytopenia, mixed cholestatic/hepatitis transaminis and coagulopathy. Five patients had maculopapular rash. Other than the finding of mild hepatitis, no bulky lesions or significant lymphadenopathy were found on CT scans of all patients. LDH and β2 microglobulin were invariably elevated. Diagnoses of EBV associated lymphoproliferative disorder were made based on demonstration of in-situ hybridization for EBV-encoded early small RNA (EBER) on bone marrow trephine, skin and liver biopsies. Three patients demonstrated the classic NK/T-cell lymphoma, nasal type phenotype (CD3-, CD4-, CD8-, CD56+), with clinical manifestation of aggressive NK-cell leukaemia at the outset, with no prior history of nasal disease. Pathology of peripheral T-cell lymphoma, nasal type phenotype (TCR rearrangement. PCR for EBV DNA performed in 3 patients showed high viral loads. Despite features of acute EBV infection, EBV capsid Ag IgM was surprising negative in all patients, while IgG was positive in all. Median survival was 55 days from time of presentation and the causes of mortality included liver failure, neutropenic sepsis and severe bleeding. Four patients received chemotherapy. Two had CHOPO regimen upfront and two had immunosuppression with etoposide, prednisolone and cyclosporin prior to full dose chemotherapy. Although mortality was uniform, those who received immunosuppression first had a longer survival. Summary/Conclusions. This disorder should always be suspected in patients who present with haemophagocytic syndrome. Its epidemiological predisposition may be accounted for by a higher prevalence of EBV infection and carrier status in the Asian population. Important pitfalls in diagnosis is the lack of serological evidence of acute EBV infection. This lack of humoral response may be the predisposing factor for clonal T-cells proliferation post-EBV infection. Reasons for this immune defect in a predominantly young and seemingly well adult population remains elusive. Immunosuppression may have a role in controlling the cytokine storm before chemotherapy is started.

Dosage-Dense CHOP (CHOP-14) Plus Rituximab for Newly Diagnosed Aggressive B Cell Lymphoma: A Prospective Multicentric Study. Grupo para el estudio de Hemopatias Malignas - Galicia

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Background. Standard front line therapy for aggressive B cell lymphoma is CHOP every 21 days associated with rituximab; CHOP every 14 days is used as an alternative improving the survival rate over of CHOP-21. Aims. To evaluate the feasibility and efficacy of CHOP-14 plus rituximab in newly diagnosed aggressive B cell lymphoma. Methods. Patients (pts) 18-75 yrs old with de novo diffuse large B cell (DLBCL) or follicular grade 3 lymphoma diagnosed between April/03 and December/05 received CHOP-14 in combination with rituximab (375 mg/m²) on day 1, and G-CSF support. In a preliminary analysis showing no adverse outcomes after 3 cycles and radiotherapy for localized or bulky disease were planned. Forty-four pts. from six institutions have entered the study, results from 43 evaluable pts (39 DLBCL and 4 follicular) are reported. Median age: 62 y (21-74), 22 pts over 60 y. Ann Arbor stage: I-II 51.2%, III 4.1%. B symptoms: 34.9%. Extranodal involvement: 58.1%. Bulky disease: 41.8%. International Prognostic Index: low/intermediate-low 62.8%, intermediate-high/high 37.2%. Results. Response: 86 pts. obtained a complete response (CR) (83.7%) and 4 pts. a partial response (PR) (9.3%); 3 pts. were not evaluable for response because of early death (7%). Medi-
an n° cycles/pt: 5 (2-8), and 14 pts. received radiotherapy. Toxicity: 29/298 cycles (9.7%) were delayed, mainly because of infections complications (17 cycles) or neutropenia (4 cycles); in 14/298 cycles (4.7%) the pt. required hospitalization, and all but one of these pts. were over 60 y. Hematologic toxicity: Anemia grade II - III WHO 23.3% and neutropenia grade III - IV WHO 23.3%; 10 pts. received an erythropoietic factor with improvement. Infectious complications grade III - IV WHO appeared in 14/298 cycles (4.7%); two pts. died from a bilateral interstitial pneumonitis without severe neutropenia and microbiological identification; none of them had received SMX-TMP prophylaxis. Other two pts. died after 2° cycle of chemotherapy from cardiovascular disease. All toxic deaths were in pts. over 65 y. Relapse: 2/56 pts (5.5%) with a median follow-up of 20 months (3-34). Survival: Seven pts. have died; 4 toxic deaths, another 2 during second-line treatment after a FR and one non-related death. The 2-years overall survival is 80.3% and progression-free survival (PFS) for pts. in CR is 79.7%. Conclusions. CHOP-14 associated with rituximab obtains a high rate of CR (83%) that also seems to be long-lasting, with only two relapses in 20 months and a 2-year PFS of 80%. Toxicity appears as reason to stop treatment especially in older pts.; only 9.7% of cycles has been delayed and, since SMX-TMP prophylaxis was initiated, no other pneumonitis have been recorded.

0699

RITUXIMAB AND ESHAP PLUS G-CSF AS AN EFFECTIVE PERIPHERAL BLOOD PROGENITOR CELL MOBILIZATION REGIMEN IN PRETREATED B-CELL NON-HODGKINS LYMPHOMA: A PRELIMINARY REPORT OF COMPARISON WITH ESHAP PLUS G-CSF

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Background. The ESAPH is reported as an excellent mobilization chemotherapy in patients with relapsed and poor-risk aggressive non-Hodgkin’s lymphoma (NHL). Rituximab added to ESAPH (R-ESAPH) has been tried as salvage therapy for relapsed and poor-risk B-cell NHL. Mobilizing stem cells following R-ESAPH should decrease time to autologous stem cell transplantation (ASCT) by making separate mobilizing chemotherapy unnecessary, while controlling a patient’s lymphoma. Aims. The aim of this study was to prospectively evaluate the efficacy of mobilization by R-ESAPH plus G-CSF regimen in relapsed or poor-risk B-cell NHL. Methods. Twenty B-cell NHL patients were enrolled. R-ESAPH plus G-CSF (Neutrgan®, Choongwae Pharma Corp., Seoul, Korea) was used to mobilize peripheral blood progenitor cells. The results were compared with those of 24 patients with NHL whose mobilizing chemotherapy was ESAPH. Results. The R-ESAPH and ESAPH groups were well balanced for age, sex distribution, prior chemotherapy cycles, number of chemotherapy regimens, and radiotherapy to the axial skeleton. Total duration of G-CSF administration was not different between the two groups. The median number of total CD34+ cells harvested per patient was 10.59 × 10^6/kg (range, 4.88-52.55 × 10^6/kg) in the R-ESAPH group and 15.34 × 10^6/kg (range, 0.04-46.0×10^6/kg) in the ESAPH group (p=0.42). The median number of CD34+ cells collected per apheresis was 4.30 × 10^6/kg (range, 0.30-21.60 × 10^6/kg) in the R-ESAPH group and 4.40 × 10^6/kg (range, 0.01-26.50 × 10^6/kg) in the ESAPH group (p=0.71). Adequate collection (total harvested CD34+ cells > 2 × 10^6/kg) was achieved in all 20 patients from R-ESAPH group and 22 of 24 (92%) patients from ESAPH group (p=0.19). Optimal collection (total harvested CD34+ cells > 5 × 10^6/kg) was obtained in 25% (19/20) of patients in the R-ESAPH group and 92% (22/24) of patients in the ESAPH group (p=0.67). Kaplan-Meier product limit estimate and log rank test revealed that the apheresis days to adequate and optimal CD34+ cell collection were not statistically different between the two groups. Thirteen patients were mobilized from R-ESAPH and 19 patients from ESAPH groups. CD34+ cell collection were not statistically different between the two groups. Thirteen patients from R-ESAPH and 19 patients from ESAPH group were mobilized with optimal collection. None of the pts. required hospitalization, and all but one of these pts. were over 60 y. Hematologic toxicity: Anemia grade II - III WHO 23.3% and neutropenia grade III - IV WHO 23.3%; 10 pts. received an erythropoietic factor with improvement. Infectious complications grade III - IV WHO were compared with those of 24 patients with NHL whose mobilization by R-ESAPH plus G-CSF regimen in relapsed or poor-risk B-cell NHL.

0700

THE OCCURRENCE OF CNS RELAPSES IN HIGH-RISK AGGRESSIVE LYMPHOMA PATIENTS TREATED WITH INTENSIFIED INDUCTION AND HIGH-DOSE CONSOLIDATION PROTOCOLS OF THE CZECH LYMPHOMA STUDY GROUP

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Background. CNS relapse of systemic NHL is usually fatal and identification of risk group and effective prophylaxis are controversial issues. Aims to analyse our cohort of high risk NHL patients in terms of CNS relapses and identify risk factors for CNS relapse. Patients and Methods. We analysed a cohort of 135 patients younger than 65 years with high-risk (age adjusted IPI 2,3) aggressive lymphomas (73% DLBCL, 15% mediastinal DLBCL, 5% peripheral T-cell, 2% mantle cell, 1% anaplastic large cell, 4% others with no burkitt and no lymphoblastic lymphoma). Patients were prospectively treated with intensified induction and high-dose consolidation protocols designed by CLSG in the period 1998 - 2004. Treatment protocols. Protocol 1: induction 3-4 courses of high-dose CHOP (cyclophosphamide 5 g/m2, doxorubicin 75 mg/m2, vincristine 1 mg, prednisolone 300 mg/m2 + G-CSF every three weeks (21 pts). Protocol 2: 3 courses of standard CHOP-21 followed by 3 courses of ESAP (26 pts). Protocol 3: 3 courses of high-dose CHOP + 3 courses of ESAP (29 pts). Protocol 4: same as protocol 3 with addition of rituximab to each cycle of chemo (59 pts). PBPCs were mobilized after 2nd or 3rd high-dose CHOP in protocol 1 and after 1st or 2nd ESAP in protocols 2,3,4. Patients in complete or partial remission after all types of induction treatment were consolidated with BEAM and ASCT. If radiotherapy was administered to initial bulk or to residual mass after chemo, CNS involvement at diagnosis was an exclusion criterion. Intrathecal CNS prophylaxis consisted of 15 mg methotrexate + 40 mg ara-C and it was recommended but not mandatory part of the protocols. Intrathecal prophylaxis received 50% of patients with median number of 3 cycles for patient (range 1-8). Results. The median age of the whole cohort was 46 years, male/female ratio 76/69, 58% had IPI 2 and 42% IPI 3. We observed 9 CNS relapses (7%), 7 on therapy, one 1 month after completion of the therapy and one late relapse following 15 months after diagnosis. Original histologies in CNS relapsing patients were DLBCL 6x, 1x mediastinal DBCL, 1x PTL, 1x FL. 5 of these patients received CNS prophylaxis (all 5 pts received i.t. MTX 15 mg+ara-C 40 mg, median 5 cycles). Median time from study entry to CNS recurrence was 5 months (range 2-15). Median survival after diagnosis of CNS relapse was 11 months (0,1-16). Nine patients (0,1-16) continued the CNS therapy and due to progression, one after 16 month in CR due to pneumonia. Evaluated risk factors for CNS progression were IPI, clinical stage, B symptoms, performance status, LDH level, intrathecal prophylaxis and type of treatment protocol. None of these risk factors were significantly predictive for CNS relapse. Summary/Conclusions. CNS prophylaxis/reapse in this cohort of high risk lymphoma patients is relatively low, but outcome of all patients is fatal. Our patients did not benefit from intrathecal prophylaxis. More precise detection of patient at risk for CNS involvement and detection of occult disease at diagnosis are needed to differentiate the treatment protocols with appropriate CNS Prophylaxis for these patients.

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0701

BURKITT AND NON-BURKITT TYPES OF CHIDHOOD B-CELL LYMPHOMAS (B- NHL) - COMPARISON OF TREATMENT RESULTS. A REPORT OF POLISH PAEDIATRIC LEUKEMIA/LYMPHOMA STUDY GROUP (PPLSSG)

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Background. The efficacy of the LMB-89 protocol for children with NHL-B has been investigated. The patients (pts) were treated in 10 onco-hematological centers of PPLLSG between 1993 and 2006. A total number of 149 children with NHL-B were included into analysis: 105 (70%) of them with B-NHL Burkitt (1 gr.) and 44 (30%) with B-NHL non Burkitt (II gr.) histopathological types (17 - Burkitt-like, 6 Large B-cell, 4 immunoblastic, 11 sMDS without interference of conditioning regimen). Median observation time was 56 months. Methods. The diagnosis was based on histomorphological investigation and supplemented with immunophenotyping. The S. Murphy staging system was used for prognostic stratification. Treatment intensity was adapted to 3 risk groups (A, B, C), according to LMB-89 protocol. Results. In both groups majority of children presented on admission advanced stage III and IV disease (75% and 15% for I gr. and 45% and 13% for II gr., respectively). Eighty-two (82%) pts were classified to B risk group. Complete remission (CR) was achieved in 94 (90%) pts with Burkitt and 42 (96%) pts with non-Burkitt types: 16 (94%) - Burkitt-like, 6 (100%) - Large B-cell, 6 (100%) - immunoblastic, 11 (100%) - others. There were 13 (9%) non-responders: 11 in I gr. and 2 in II gr. Eight early deaths were observed: 7 in I gr. (4 advanced tumour in diagnosis, 1 acute renal failure+peritonitis, 1 St.aureus sepsis+varicella, 1 multiorgan failure+ myelosuppression) and 1 in II gr. (advanced tumour in diagnosis). 11 relapses were observed: 7 in I gr. and 4 in II gr. (1 Burkitt like, 1 - immunoblastic, 2 ' others). 8 pts died after RC: 5 in I gr. due to disease relapse, 3 in II gr. (2 relapse, 1 toxicity related death (lungs failure)). Probability of EFS was 0.86 for all pts (in previous study 0.75). The EFS of I + II, III and IV stages were: 0.95, 0.87 and 0.71 respectively (p=0.02). The EFS for B-NHL Burkitt was 0.83 (in previous study 0.81) and for non-Burkitt 0.87. Conclusions. The treatment intensity of children with B-NHL, especially with Burkitt type has improved in comparison to previously reported observations. Higher EFS and overall survival of B-NHL, including Burkitt type, could be achieved thanks to quick diagnosis after first tumour clinical symptoms and an improvement of intensive supportive care (adequate blood product substitution, regular infection specific prophylaxis, prophylaxis of MTX therapy monitoring) for therapy toxicity elimination (compared with previous study). The worst results are observed in children with bone marrow involvement and in those with large tumor burden at diagnosis in two examined pts groups.

0702
HAEMATOLOGICAL AND EXTRAHAEMATOLOGICAL MALIGNANCIES AFTER AUTOLOGOUS TRANSPLANTATION FOR LYMPHOPROLIFERATIVE DISEASES
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Background. Secondary myelodysplastic syndrome (sMDS), acute myeloid leukemia (sAML) and solid neoplasia are serious complications after high-dose chemotherapy followed by autologous bone marrow (BMT). Aims. We wanted to compare the clinical features and prognosis of T-cell lymphoma with that of diffuse large B-cell lymphoma (DLBL). Previous published papers, however, gave no consistent data regarding the differences between two lymphomas and enrolled many various cell types as well as different treatments resulting in obscure observation for a certain lymphoma type. Methods. Patients with PTCLu and DLBL were selected when their first-line chemotherapies were CHOP. Clinical data were collected through retrospective review of medical records. Progression-free survival (PFS) and overall survival (OS) were calculated from the first day of CHOP chemotherapy.

Results. From Nov 1997 to Sep 2005, 397 patients received CHOP as first-line chemotherapy. Among them, 290 and 107 patients were classified as B-cell and T/NK-cell lymphoma, respectively. Total 238 patients with DLBL and 40 patients with PTCLu were analyzed in this study. Gender (p=0.530) and age at diagnosis (p=0.750) were not different between patients with PTCLu and DLBL. Patients with PTCLu were more in advanced stage (p=0.003) and had more B symptoms (p=0.007). The number of extranodal involvement (p=0.114), LDH (p=0.816), IPI (p=0.151), overall response rate (89.1% vs. 40%, p=0.544) and time to best response (3.89 vs. 5.06 months, p=L201) were not different between two groups. However, complete remission (CR) rate to CHOP was higher in DLBL (75.2% vs. 60%, p=0.045). The progression rate (21% vs. 47.5%, p=0.001) and frequency of second-line chemotherapy (24.7% vs. 40%, p=0.017) were higher in PTCLu. PFS was shorter in PTCLu than in DLBL (24.87 months vs. not reached; p=0.02). The EFS for B- NHL Burkitt was 0.83 (in previous study 0.81) and for non-Burkitt 0.87. Conclusions. The treatment intensity of children with B-NHL, especially with Burkitt type has improved in comparison to previously reported observations. Higher EFS and overall survival of B-NHL, including Burkitt type, could be achieved thanks to quick diagnosis after first tumour clinical symptoms and an improvement of intensive supportive care (adequate blood product substitution, regular infection specific prophylaxis, prophylaxis of MTX therapy monitoring) for therapy toxicity elimination (compared with previous study). The worst results are observed in children with bone marrow involvement and in those with large tumor burden at diagnosis in two examined pts groups.
an, both not reached; p=0.4019; Figure 1B). Summary/Conclusions. FTCLs showed more frequent relapse rate and poor PFS after CHOP chemotherapy, in spite of similar response rate to first-line CHOP chemotherapy compared with DLBL. Responses and relapse rate to second-line chemotherapy were similar and OS was not different in both groups.

0704
RITUXIMAB-CHOP AND RADIOThERAPY FOR PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA: AN UPDATE
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Background. MACOP-B or even chemotherapy (CT) with consolidation high dose therapy with autologous stem cell support (HDT-ASCT) have been considered superior to CHOP in PMLBCL. However, in the absence of randomized trials, there is no established optimal treatment for these patients. Recent data have shown that R-CHOP is superior to CHOP in patients with diffuse LBCL, so that it is rapidly becoming the new standard of care for this subtype of aggressive lymphoma. In younger, intermediate/high-risk patients with aggressive lymphomas HDT-ASCT was superior to conventional CT in the pre-rituximab era, but its role in the era of rituximab is unclear. Thus the role of R-CHOP in the particular case of PMLBCL, which usually affects young patients, is not well established yet. Aims. The evaluation of the efficacy of R-CHOP±RT in PMLBLC and the comparison of this approach with CHOP±RT, administered to historical controls. Patients and Methods. Between 1994 and 2005, 62 patients with PMLBCL were treated in 4 participating centers. R-CHOP displaced CHOP in the treatment of PMLBCL at a given timepoint in each center. Thus 28 consecutive patients who received R-CHOP, were compared to 34 consecutive historical controls. Although R-CHOP patients were treated up to that point. Results. The median age of the patients was 32 years (17-63) and 42/62 (68%) were females. Age-adjusted IPI was ≥2 in 39% and 46% of patients who received R-CHOP and CHOP respectively (p=0.61). All individual IPI parameters as well as B-symptoms were also balanced between the two groups. The complete response (CR) rate was 100% for R-CHOP±RT vs 64% for CHOP±RT (p=0.001). All relapses after CHOP occurred within 22 months from diagnosis. No relapse has been recorded after R-CHOP, while a single patient with CR but persistent PET abnormality underwent stem cell transplantation and was considered as failure. The 3-year failure free survival (FFS) was 95±4% vs 51±3% for patients who received R-CHOP±RT vs CHOP±RT (p=0.001). Within the subgroup of patients with L/LI risk IPI the corresponding 3-year FFS rates were 100% vs 57±11% (p=0.008), while they were 89±10% vs 44±12% (p=0.04) among patients with HI/H risk IPI. The 3-year event free survival (EFS) for all patients was 91±6% vs 51±8% (p=0.003). The 4-year overall survival was 96±4% vs 66±8% (p=0.03), while the 4-year lymphoma specific survival was 100% vs 66±8% (p=0.008). Conclusions. R-CHOP and RT provided impressive results with only one failure and lymphoma-related deaths among 25 patients. In comparison to CHOP-treated historical controls, highly significant differences in favor of R-CHOP were recorded in terms of CR, FFS, EFS, and LSS rates. Overall survival was also improved. Based on these results we continue to treat PMLBCL patients with R-CHOP and RT. The need for more aggressive strategies, such as MACOP-B or ASCT, is therefore questionable. Whether RT is needed after R-CHOP, especially when post-chemotherapy PET-scan is available, should be investigated.

0705
TREATMENT STRATIFICATION ACCORDING TO EARLY RESPONSE TO MEGA-CHOP, BASED ON CT AND GALLIUM 67 SCAN WITH OR WITHOUT IFE SALVAGE THERAPY FOLLOWED BY AUTOLOGOUS STEM CELL TRANSPLANTATION IN PATIENT WITH POOR PROGNOSIS AGGRESSIVE LYMPHOMA
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Background. Patients with IPI, ≥3 large cell lymphoma have a poor outcome with long term survival lower than 50%. Evaluation of response only with CT scan shows often residual masses which can be tumoral or fibrotic. Gallium 67S discriminate better these two situations and therefore can help to decide further strategies. Aim. To assess the efficacy of PBSCT in patients with poor prognosis aggressive NHL according to previous early response to Mega-CHOP evaluated with CT & Ga67S. Patients and Methods. Inclusion criteria were: Ga67S positive large cell b lymphoma with IPI score > 2 or IPI <2 with high β2 microglobulin or peripheral T cell lymphoma (PTCL), except ALK+ anaplastic lymphoma regardless of IPI. Patients were evaluated after 3 cycles of Mega-CHOP. Those in CR (CT scan, Ga67S negative) or uCR (CT scan positive, Ga67S negative) received a 3rd Mega-CHOP followed by BEAM and PBSCT. Those with positive Ga67S received IFE or ESHAP (x2) regimen followed by BEAM and PBSCT. Patients with refractory disease (RD) were dropped from the study. Since 2001, 112 patients have been registered and 87 have finished the treatment. Median age was 52 years (20-67 years) and 49% were males. Seventy one (72%) had a DLBCL, 8% were a grade 3 FL and, 24 (21%) PTCL. Sixty two (88%) had IPI > 2, and 12% IPI 1. Doses were for Mega-CHOP : Cy 1.5 g/m2, ADR 65 mg/m2 and VCR 2 mg on day 1 and Pred 60 mg/m2 days 1-5 on a 21 day schedule and for IFE : ifosfamide 10 g/m2 and VP16 900 mg/m2 (days 1-3) with Mesna. Results. After 3 Mega-CHOP, 47 patients (42%) were considered on CR or uCR due to a negative Ga67S, 46(41%) were on PR and 18 (16%) were refractory. One patients were early deaths. After IFE 18(46%) achieved CR, 19 (41%) PR and 9 (20%) progressed. Overall, 87 patients received PBSCT and are valuable for response. Thirty one patients (28%) died, 23 (21%) due to lymphoma and 5 (7%) due to toxicity. With 36 months of median follow-up (8 to 69 months), 81 patients are alive, 67 (60%), disease free. Five-year overall survival according to clinical response and Ga67S after the 3 initial Mega-CHOP were 70% (CR, PR and Ga67S neg.), 67% (CR, PR Ga67S pos.) and 57% (failure Ga67S pos.), respectively (p=0.0004). In the univariate analysis, the significant variables associated with outcome were clinical response combined with positive/negative Ga67S after the initial therapy (p=0.0004) and disease status at ASCT (p=0.01). Conclusion. Our preliminary results suggest that early salvage therapy can overcome the poor outcome of patients with bad prognosis aggressive lymphoma. Moreover, this early evaluation could identify patients with poor prognosis who only need a short treatment (4 Mega-CHOP+PBSCT).
Preliminary Results from a Phase II Study of Lenalidomide Monotherapy in Relapsed/Refractory Aggressive Non-Hodgkin's Lymphoma


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Background. Lenalidomide (Revlimid®) is an immunomodulatory drug of the IMiD class, recently approved in the US for myelodysplastic syndromes associated with a deletion 5q (5q)-cytogenetic abnormality that also has activity in multiple myeloma, chronic lymphocytic leukemia and cutaneous T-cell lymphoma. Thalidomide, a less potent IMiD, has activity in non-Hodgkin's lymphoma as both monotherapy and in combination with rituximab. Aim. To assess the safety and efficacy of lenalidomide monotherapy in subjects with relapsed/refractory aggressive non-Hodgkin's lymphoma (NHL). Methods. Subjects with relapsed/refractory aggressive NHL following > 1 prior treatment regimen with measurable or until disease progression. Response and progression are evaluated using the IWLR methodolgy. Tolerated or until disease progression. Response and progression are evaluated using the IWLRC methodology. There is lack of evidence of mide monotherapy in subjects with relapsed/refractory aggressive NHL are encouraging. Grade 4 general malaise.


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Background. Correct staging is important for the appropriate treatment in lymphoma patients. Most cancers, including lymphomas, metastize to other organs or spread, so an accurate staging is a key tool in the evaluation of patients with lymphoma. Many authors in these last years have shown the importance of FDG-PET/CT analysis at diagnosis of lymphomas and the differences according to histologic subtypes. Aim. The III (Italian Lymphoma Intergroup) evaluated: 1) the role of FDG-PET/CT versus CT scanning in the staging of Non-Hodgkin's lymphoma, 2) the significance of FDG-PET/CT according to histologic subtypes, 3) the ability of FDG-PET/CT in showing extranodal localizations. Methods. We have retrospectively analysed at diagnosis 105 patients (50 male, 52 female) with both FDG-PET/CT and conventional CT scanning. The histologic subtypes were: diffuse large B-cell lymphoma (LBCL) 49 pts (47%), follicular lymphoma (FL) 37 pts (35%), marginal zone lymphoma (MZL) 7 pts (6%), mantle cell lymphoma (MCL) 4 pts (4%), Burkitt and Burkitt-like lymphoma (BL) 3 pts (3%), primary mediastinal B-cell lymphoma 2 pts (2%), other lymphomas (small lymphocytic, peripheral T-cell, extranodal) 3 pts (3%). The PET-CT evaluation (GE, Discovery, LS) were performed 60 min. after the i.v. injection of 18F-FDG (5.5 MMBq/kg) with a whole-body acquisition with a field of view extending from the head to the upper part of the thighs. All patients fasted for at least 8 hours prior to FDG injection and had a glucose level < 120 mg/dl. Results. We have evaluated nodal (18) and extranodal (12) stations. Considering all cases, the agreement between FDG-PET/CT and CT scanning was 89% in nodal stations and 95% in extranodal ones, while discordance was 9% (7% toward PET/CT and 2% toward CT), and 5% (4% toward PET/CT and 1% toward CT) respectively. The percentage was similar in all the different histologic subtypes. The extranodal localizations in which there were more discordances were spleen (7 pts), bone (3 pts), and brain (2 pts). FDG-PET/CT upstaged 27/105 pts (26%) and for 17% of pts the upstaging modified therapy (0 → III-IV in 4 pts (4%), I → III-IV in 3 pts (3%), II → III-IV in 10 pts (10%). The FDG-PET/CT downstaged only 9/105 pts (9%).: I → 1 in 1 pts (1%), III-IV → I in 5 pts (5%), I → 0 in 3 pts (3%). Conclusions. FDG-PET/CT and CT scanning are concordant, for nodal and extranodal localizations, in staging of Non-Hodgkin's lymphomas. FDG-PET/CT shows more nodal localizations (7%) and extranodal localizations (4%) than CT scanning. There isn’t s substantial difference in concordance between FDG-PET/CT and CT scanning according to the various histologic subtypes. It is important to have FDG-PET as baseline for early and late evaluation during and after therapy. FDG-PET/CT is essential for staging lymphomas also as exclusive method.


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EXTRA-NODAL NON-HODGKIN LYMPHOMAS AT A COMPREHENSIVE CANCER CENTRE IN PORTUGAL

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Background. Non-Hodgkin Lymphomas (NHL) can arise within any organ in the human body. Extra-nodal NHL account for about 25% of the total cases of lymphoma diagnosis. Aim. To evaluate the relative frequency of adult extra-nodal NHL and their absolute and relative outcome in the last five years in a Portuguese comprehensive cancer centre. Methods. Retrospective analysis of a cohort of adult patients with histologically confirmed diagnosis of extra-nodal NHL between January 2001 and December 2005 treated at Instituto Português de Oncologia Porto. A lymphoma was classified as extra-nodal when the biggest lymphomatous mass was located outside a lymph node and patient's chief complaints were attributable to it. Patients with Burkitt's lymphoma and lymphoblastic lymphoma were excluded. Data on demographics, histology, stage according to Ann Arbor system, known prognostic factors and treatments performed were collected. Descriptive analysis was performed as appropriate for each variable. Overall survival and survival as per anatomical location were analysed. Significance of prognostic factors on survival were evaluated by Log Rank test. Results. A total of 119 patients were identified. The mean age at diagnosis was 58.4 years (range: 19-96). Fifty two percent of patients were male. The most prevalent locations were the stomach with 54 cases and the skin with 15 cases. All other locations were represented with less than 10 cases each. Treatment differed according to the extra-nodal location and to the specific patient and disease characteristics. Median time of follow up was 20 months. The median survival was not reached either for the whole population or for any of the more prevalent locations. Survival at 24 months was 70%. Overall 32 deaths were registered. In univariate analysis, survival was significantly worse for age above 60 years (n=119, p=0.01), ECOG of 2 or more (n=117, p<0.0001), LDH above normal (n=109, p<0.0001), albumin below normal (n=110, p=0.0001) and Ann Arbor stage III or IV (n=116, p=0.0001). Summary/Conclusions. In this single institution series, as with most reported series, the most prevalent locations were the stomach with 54 cases and the skin with 15 cases. All other locations were represented with less than 10 cases each. Treatment differed according to the extra-nodal location and to the specific patient and disease characteristics. Median time of follow up was 20 months. The median survival was not reached either for the whole population or for any of the more prevalent locations. Survival at 24 months was 70%. Overall 32 deaths were registered. In univariate analysis, survival was significantly worse for age above 60 years (n=119, p=0.01), ECOG of 2 or more (n=117, p<0.0001), LDH above normal (n=109, p<0.0001), albumin below normal (n=110, p=0.0001) and Ann Arbor stage III or IV (n=116, p=0.0001).
100% complete remission rate after median of 30 days and 72% survival rate after median 60 days of follow-up. Main toxicities included hyperleukocytosis, hepatic toxicity and APL differentiation syndrome. The results imply that arsenic trioxide is an effective anti-leukemia and anti-angiogenesis agent in new cases of APL.

0712

**SALVAGE TREATMENT WITH HIGH DOSE THERAPY AND PBST IN HIGH GRADE NHL - FROM THE GILS (GRUPPO ITALIANO STUDIO LINFOMI)**

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Background. The inclusion of high-dose therapy and PBSCT as salvage treatment in patients with HG-NHL is generally planned in designed protocols. However, it is generally difficult to apply the therapy to all expected patients due to several reasons, including the disease progression which leads frequently to change the assigned therapy, or to compliance of patients with high-dose therapy, or to difficult to obtain an adequate number of CD34 cells. Aims. We focus the study on a population of patients with HG-NHL addressed to salvage treatment, including high-dose therapy. The analysis is finalised to show if there are differences on survival according to an intention to treat (ITT) between those addressed to a conventional (CT) or to a high-dose (HDS) salvage treatment, either in patients relapsed or in those primarily resistant to previous therapy. Methods. Since 1985 up to 2006, 122 patients with HG-NHL completed the assigned therapy according the underlying protocols; 256 patients of age < 60 years were assigned to a salvage program either because primarily resistant (103=44%) or relapsed (153=56%). Based on ITT analysis we evaluated CT or high-dose therapy HDS in 181 valuable patients. One hundred twenty two patients were assigned to CT (67%) and 60 to HDS (33%). Among the 60 patients addressed to HDS 38 (63%) did the planned therapy and 22 patients were assigned to CT (67%) and 60 to HDS (33%). Among the relapsed (133=56%). Based on ITT analysis we valuated CT or high-salvage program either because primarily resistant (103=44%) or relapse (14%) CR with CT and 30% with HDS; instead of young patients with HG-NHL addressed to salvage treatments for disease progression, occurred in 70% and 36% of relapsed patients 14% CR with CT and 30% with HDS; instead of young patients with HG-NHL addressed to salvage treatments for disease progression, occurred in 70% and 36% of refractory patients treated by CT and HDS, respectively. Relapse following salvage therapy, occurred in 70% and 36% of refractory patients treated by CT and HDS, respectively. The OS of relapsed patients according to ITT analysis show a better significance of survival according to two median survival, 6 and 10 months for CT and HDS, respectively; the 3 years survival of refractory patients according to ITT analysis show 8 months for CT and 10 months for HDS, respectively. Relapse following salvage therapy, occurred in 70% and 36% of refractory patients treated by CT and HDS, respectively. The OS of refractory patients according to ITT analysis show 8 months for CT and 10 months for HDS, respectively; the 3 years survival of refractory patients according to ITT analysis show 8 months for CT and 10 months for HDS, respectively; the 3 years survival is 38 and 60 months for CT and HDS, respectively (p=0.056). The OS of relapsed patients is 26 months median, 21 and > 84 months for CT and HDS, respectively. The 3 years survival is 38 and 70%, for CT and HDS, respectively (p=0.029). The analysis for therapy efficacy done show a better significance of survival according to two arms of treatment (p=0.025). Conclusions. Our study demonstrated that HDS in relapsed patients with HG-NHL exerts a better outcome than CT, whether the same result could be obtained in refractory patients should be further investigated by prospective studies even if a positive trend could be already disclosed in this subset of patients.

0713

**THE EFFICACY OF RITUXIMAB PLUS IFOSFAMIDE SECOND LINE APPROACH IN RELAPSED/ REFRACTORY NON HODGKIN LYMPHOMA**

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Background. relapsed high grade NHL is a very aggressive pathology characterized by a worse prognosis. High dose therapy followed by peripheral blood stem cell (PBSC) rescue is, to date the gold standard therapy: patients who undergo transplant in complete response have a good CD 34 harvest and c) represents an effective in vivo purging agent.

0714

**SPLENIC MARGINAL ZONE LYMPHOMA: A CLINICOPATHOLOGICAL STUDY IN 16 PATIENTS**

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Background. Splenic marginal zone lymphoma (SMZL), recently characterized in the WHO classification of lymphoid tumors, is a rare disorder comprising less than 1% of lymphoid neoplasms. Thus, it is not to collect a big group of patients, and only few series have been published. We analyse SMZL from our community, from a clinical, biological, and pathological point of view, and to compare our findings with those reported in bibliography. Methods. We retrospectively studied 16 cases of SMZL, who were diagnosed in our community over the last eight years (mean age 59, range 44-75; 11 males, 5 females). Analysed data included clinical features at presentation and evolution, analytical, morphological and immunophenotypic findings: presence of chromosomal abnormalities; frequency of extranodal and bone marrow (BM) involvement; infiltration pattern on marrow biopsy; transformation to aggressive lymphoma; response to chemotherapy, and survival rate. Results. Patients more frequently presented with splenomegaly (94%), BM involvement (87.5%), BM reticulin myelofibrosis (75%), peripheral blood involvement (62.5%), elevated β2-microglobulin (61,5%), abdominal discomfort secondary to splenomegaly (56%), anaemia + thrombocytopenia (50%) and ‘villous’ lymphoid morphology (50%). Less frequent were the existence of chromosomal aberrations (37,5%), β symptoms (31%), elevated lactate dehydrogenase (LDH) (25%), hepatomegaly (19%), positive DAT (18%), and peripheral lymphadenopathies (13%). Lymphomatous-cell immunophenotyping showed positivity to CD20 (100% of analysed cases), CD22 (91%), CD79b (75%), FMC7 (75%), CD5 (45%), and CD23 (40%); only two cases were CD25+ or CD11c+, one expressed CD10+, and BM CD103-positive. Nodular pattern was the most frequent pattern of marrow invasion (6/14 patients), while intrasinusal BM infiltration was rarely found (1/14). Extranodal involvement was seen in two patients (12,5%), as specific pleural effusion and ascites. Only one patient (6%) presented with a serum M component (IgA). A chromosomal abnormality was detected in three patients, two of them chromosome 7-related. Transformation to aggressive lymphoma occurred in three cases. Finally, 4 patients have died, and mean survival is 44 months (range 16-93). Conclusions. We did not find significant differences between this serie and those reported in bibliography. Nevertheless, our
patients rarely showed an intrasinusoidal BM infiltration pattern or a sex cord-like component, both reported features of this type of lymphoma. As mentioned by other authors, CD5-positive SMZL cases seem to be more common that previously thought.

0715
IS DOUBLE-BALLOON ENDOSCOPY USEFUL AND NECESSARY FOR THE EVALUATION OF SMALL INTESTINE INVASION IN PATIENTS WITH NHL?
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Background and Aims. It is difficult to diagnose correctly the invasion of non-Hodgkin lymphoma (NHL) in small intestine. Recently, double-balloon endoscopy (DBE) (Fujinon Co. Ltd., Tokyo) has been produced and spread as a new and easy-to-use method of endoscopy for whole small intestine. In this study, we studied the usefulness of DBE in patients with NHL for diagnosis of invasion in small intestine. Patients and Methods. From February 2005 until January 2006, DBE was underwent in twelve patients with NHL. Six patients were systemic NHL, five patients were gastric NHL, and one patient was rectal NHL. They were seven males and five females, with an average age of 63.6 years (range: 48 to 78). The pathological findings were 7 diffuse large B cell lymphoma (DLBCL), 3 follicular cell lymphoma (FCL), 1 Mahtoma, 1 mantle cell lymphoma, and 1 IPSID. DBE was basically twice done in every case both from mouth and anus on different day as possible. All patients were also underwent biopsy at DBE. Results. DBE was safely underwent in all 12 patients. Characteristic endoscopic findings of small intestine were revealed in six patients with NHL. However, in only 4 patients, biopsy specimen showed positive. In the rest two patients, there was no pathological finding of NHL, which was considered due to chemotherapy at the previous hospitals. Both of two FCL cases had endoscopic and pathological findings in the small intestine. They were diagnosed intestinal perforation because of chemotherapy at the previous hospitals. If they were noticed that their NHL was invasive in small intestine, we were able to speculate their small intestine might be perforated after chemotherapy. Only 2 gastrointestinal NHL patients had small intestinal lesion. On the other hand, 3 systemic NHL patients had also invasion in small intestine. Especially, IPSID patient was diagnosed with only DBE. Aspiration pneumonia was happened in one patient. Other severe complication was not found. Conclusions. DBE was valuable for diagnosis of NHL invasion in small intestine. DBE must be selected before chemotherapy for NHL.

0716
NODAL VS. PRIMARY EXTRANODAL DIFFUSE LARGE B-CELL LYMPHOMAS: A COMPARISON OF PRESENTING FEATURES, RESPONSE TO TREATMENT AND OUTCOME
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Background. Diffuse large B-cell lymphomas (DLBCL) represent the commonest subtype of non-Hodgkin's lymphomas (NHL) in Western countries, comprising 30% of the total. Marked heterogeneity in aspects of morphology, immunophenotype and genetics is their main characteristic. Approximately 80% of them are of primary extranodal origin. It has been proposed that the distinction between DLBCL could be regarded as two distinct clinical entities, since molecular differences between them suggest a different genetic origin. Aim: To assess the main clinical presenting features, response to treatment and outcome of a large number of patients with DLBCL according to the primary site of origin, nodal or extranodal. Methods. Between 1976 and 2005, 398 consecutive patients with DLBCL were treated in our department. CHOP and CHOP-like regimens were administered to a total of 358 (88.8%) patients, 60 (17%) of which received additionally radiotherapy, 74 (21%) rituximab and 35 (9.9%) both radiotherapy and rituximab. Patients were divided in two groups (DLBCL A, 2 follicular cell 47.2%), patients with DLBCL of primary nodal origin and group B, that consisted of 210 (52.8%) patients with DLBCL of primary extranodal origin. Patients’ characteristics (gender, age, stage, IPI, presence of B symptoms, bulky disease and bone marrow infiltration), the kind of treatment (chemotherapy, rituximab, radiotherapy) and response rates were compared between the two groups. Disease-free survival (DFS), overall survival (OS) and failure-free survival (FFS) were estimated according to the Kaplan-Meier method. Differences in survival rates were assessed using the log-rank test. Results. Group B patients presented with early stage disease (I-II, no bulky disease), low IPI (0-1), no B symptoms, and no bone marrow infiltration with a significantly higher frequency than group A patients (83.8% vs. 45.7%, 62.8% vs. 39.9%, 20% vs. 34.6%, 2.4% vs. 12.2% respectively, p<0.003). Patient distribution according to the kind of administered treatment, was not different between the two groups (p>0.05). Median follow-up time for groups A and B was 55 (1-425) and 56 (1-426) months respectively (p>0.05). On an intention-to-treat basis, complete response rates were similar in groups A and B (81.9% vs. 84.8% respectively, p=0.05). Actuarial 5-year DFS rate was significantly higher in group B compared to group A (80% vs. 68.3% respectively, p=0.006). Actuarial 5-year OS and FFS rates were not significantly different between groups A and B (71.3% vs. 70.8% and 55.2% vs. 49.5% respectively, p>0.05). Conclusion. In our study, patients with DLBCL of primary extranodal origin demonstrated a greater probability of presenting clinical features and a higher DFS rate than patients with nodal DLBCL. Nevertheless, OS and FFS rates did not seem to be affected by the primary site of origin.

0717
THE LATE CARDIOTOXICITY OF DOXORUBICIN CONTAINING REGIMENS IN THE TREATMENT OF MALIGNANT LYMPHOMAS
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Background. Chronic cardiotoxicity of doxorubicin occurs later than one year after completion of the chemotherapy and it represents a serious late treatment related complication. Aims. To determine the occurrence of late clinical and subclinical doxorubicin cardiotoxicity and to compare the cardiopulmonary performance status in the patients surviving more than 5 years after primary treatment for lymphoma. Methods. 96 patients with Hodgkin’s and non-hodgkin’s lymphoma treated in the period 1995 – 2000 at our department were consecutively included in the present study. Male/female ratio 47/49, median age 49 (23-79), medical age 41 (23-79), median follow-up 6 years (5-10). The maximum cumulative dose of doxorubicin (CD DOX) used in the treatment protocols was 377+147 (median 300, 50-880) mg/m2. 32 (33%) of the patients received another treatment after primary regimen for high risk disease at the time of diagnosis or for later relapse. Patients were examined by rest echocardiography before initial treatment, after its completion and at the minimum of 5 years follow-up in the survivors. Dynamic stress echocardiography and cardiopulmonary exercise test were performed during control examination. Decline of left ventricular ejection fraction (LVEF) below 50%, progression of decline of LVEF >10% compared to baseline value and drop of peak oxygen consumption pVO2<20 mL/kg/min were considered as pathological. Doppler parameters of left ventricular diastolic function and index of global left ventricular function (myocardial performance index, MPI) were evaluated too. Results. Clinical signs of cardiotoxicity were observed in 4% of pts, subclinical cardiotoxicity in 51%, impairment of diastolic function was present in 38% pts and a pathological value of MPI in 31% pts. A stress increment of EF was 13±4%, rise of decline of LVEF > 10% compared to baseline value and drop of pVO2<20 mL/kg/min was considered as pathological. Doppler parameters of left ventricular diastolic function and index of global left ventricular function (myocardial performance index, MPI) were evaluated too. Results. Clinical signs of cardiotoxicity were observed in 4% of pts, subclinical cardiotoxicity in 51%, impairment of diastolic function was present in 38% pts and a pathological value of MPI in 31% pts. A stress increment of EF was 13±4%, rise of decline of LVEF > 10% compared to baseline value and drop of pVO2<20 mL/kg/min was considered as pathological.

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Background. Activated γδ T cells possess intrinsic cytolytic antitumour activity in different carcinomas, sarcomas, myeloma and leukemia. Activated γδ T cells express antigens CD25+ and CHOP-like regimens were administered to a total of 353 (88.8%) clinical presenting features, response to treatment and outcome of a prospective study. Between 1976 and 2005, 398 consecutive patients with NHL were treated in our department. CHOP and CHOP-like regimens were administered to a total of 358 (88.8%) patients, 60 (17%) of which received additionally radiotherapy, 74 (21%) rituximab and 35 (9.9%) both radiotherapy and rituximab. Patients were divided in two groups. Disease-free survival (DFS), overall survival (OS) and failure-free survival (FFS) were estimated according to the Kaplan-Meier method. Differences in survival rates were assessed using the log-rank test. Results. Group B patients presented with early stage disease (I-II, no bulky disease), low IPI (0-1), no B symptoms, and no bone marrow infiltration with a significantly higher frequency than group A patients (83.8% vs. 45.7%, 62.8% vs. 39.9%, 20% vs. 34.6%, 2.4% vs. 12.2% respectively, p<0.003). Patient distribution according to the kind of administered treatment, was not different between the two groups (p>0.05). Median follow-up time for groups A and B was 55 (1-425) and 56 (1-426) months respectively (p>0.05). On an intention-to-treat basis, complete response rates were similar in groups A and B (81.9% vs. 84.8% respectively, p=0.05). Actuarial 5-year DFS rate was significantly higher in group B compared to group A (80% vs. 68.3% respectively, p=0.006). Actuarial 5-year OS and FFS rates were not significantly different between groups A and B (71.3% vs. 70.8% and 55.2% vs. 49.5% respectively, p>0.05). Conclusion. In our study, patients with DLBCL of primary extranodal origin demonstrated a greater probability of presenting clinical features and a higher DFS rate than patients with nodal DLBCL. Nevertheless, OS and FFS rates did not seem to be affected by the primary site of origin.


E0710 RITUXIMAB-CHOP EVERY 14 DAYS IN NAIVE PATIENTS WITH DIFFUSE B-LARGE CELL LYMPHOMA

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Introduction. Some studies have shown that patients with aggressive lymphoma may benefit from dose intensified schedules as CHOP-14. The addition of rituximab (R) improves response rate and survival. The support with G-CSF in dose intensification regimes may provide a good complementation of courses and advantage compared with schedules standard-dose as R-CHOP Purpose: To evaluate the efficacy of R-CHOP-14 in naive patients with diffuse B- large cell lymphoma (DLBCL) (REAL classification). Design: observational, prospective and multicentric trial in a consecutively and previously untreated patients diagnosed of DBLC CD20+. Exclusion criteria: HIV positivity, other malignancies and CNS involvement. Patients and Methods. Since June 2005 to January 2006, 51 patients were included in an R-CHOP regimen administered every 14 days (8 courses). At baseline assessment: clinical and physical exam, blood counts, serum and urine biochemistry, albumin, β-2-microglobulin and LDH level, body span, bone marrow biopsy. Patients were classified according ECOG, clinical stage and IPI. All patients receiving prophylaxis with haematopoietic factors. Re-staging studies were performed every 4 cycles. Responses were classified as complete remission (CR), partial remission (PR), and non response (NR). Statistical analysis: Overall survival (OS), relapsed Free survival (RFS). Survival analysis was performed using Kaplan-Meier and Cox regression. Results. Mean age 54 y (20-78), male 66.6%. ECOG 0(21), 1(21), 2(3), 3(3). B symptoms 55%. IPI score 0(3), 1(10), 2(11), 3(13), 4(7), 5(1), stage I(3), II(9), III(12), IV(27). Bulky disease 12 patients, only extranodal location 4, with extranodal location 31, haemoglobin<10 g/dL 12, albumin<3 g/dL 15, high LDH 34, high β-2-microglobulin 30. After 4 cycle: 47 valuable patients (92.1%); response: 491(94.1%), 12 CR (25.5%), 31 PR (65.9%), 4 NR. After 8 cycle: 34 valuable patients (66.6%), 32 CR (94.1%), 1 PR, 1 NR. 5 patients have relapsed (5.9%) and 12 died (23.6%) (progression 6, infection 5, > 70 years 4). Adverse events 187 episodes: myelotoxicity 72% (grade 3-4 neutropenia and thrombocytopenia were observed in
80% and 16% respectively), infection 12%, gastrointestinal (5%), others (10%). OS was 3 months and mean PFS 40 months. Conclusions. A high response rate to R-CHOP 14 in adults naive DBLC patients (94.1%) was observed in this study with acceptable toxicity. No differences in response were observed according to age groups but higher myelotoxicity and adverse events was present in older than seventy

0721
INFLUENCE OF SERUM VASCULAR ENDOTHELIAL GROWTH FACTOR UPON TUMOR PROGRESSION IN PRIMARY SMALL INTESTINE LYMPHOMA
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The aim of this study was attempted to clarify the relationship between the serum vascular endothelial growth factor (VEGF) and clinicopathological characteristics in patients with primary small intestine lymphoma. Materials and Methods. The 50 patients with primary small intestine lymphoma ranged age was from 34-72 years (mean, 56 years) and included 21 men and 9 women. All cases satisfied the criteria for primary gastrointestinal lymphoma. All histologic materials were obtained by endoscopic biopsy, surgery. Immunophenotyping were assessed by monoclonal antibodies. After dividing the cases into either B-cell or T-cell phenotypes, B-cell lymphomas were classified according to the Revised European-American Classification of Lymphoid Neoplasms. The stage of tumor was classified according to modification of Musshoff et al. of the Ann Arbor staging system. Serums were assayed for VEGF quantitative sandwich ELISA. The minimum detectable level of VEGF was 9 pg ml-1. Analysis of differences in VEGF levels between two groups of various prognostic factors was performed with Mann-Whitney U test. The posttreatment survival probability of the patients was calculated by Kaplan-Meier method in all 30 patients and compared with the log-rank test. Results. The serum VEGF levels were significantly higher in patients with colorectal and/or gastric involvement than those who did not (715±0.17 pg ml-1 vs. 314±0.2 pg ml-1, p<0.001), in patients with diffuse infiltration under macroscopic than those who did not (789±0.15 pg ml-1 vs. 404.0.15 pg ml-1, p<0.001), in patients with high grade histology than those who did not (767±0.19 pg ml-1 vs. 367±0.06 pg ml-1, p<0.001) and in patients with perforation than those who did not (779±0.2 pg ml-1 vs. 589±0.14 pg ml-1, p<0.001). Those patients with MALT type tumors, less advanced stage of disease, B-cell phenotype had significantly lower serum VEGF levels. The high serum VEGF levels were significantly associated with poor survival. Conclusion. The high serum VEGF levels (>575 pg ml-1) appears to have a poor prognosis among patients with primary small intestine lymphoma. Our study may provide a basis for the better evaluation of biological characteristics and a new therapeutic strategy.

0722
D-PACE REGIMEN: AN EFFECTIVE CHEMOTHERAPY REGIMEN TO CYTROREDUCE REFRACTORY AND EXTENSIVELY PRETREATED LYMPHOMAS BEFORE ALLOGENEIC STEM CELL TRANSPLANTATION
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Background. Lymphoma patients who relapse after several lines of chemotherapy or after autologous stem cell transplantation (SCT) have a very poor prognosis with a long-term survival <15%; currently there is no standard salvage chemotherapy regimen for this subset of patients. Aims. The objective of the study was to assess the efficacy of D-PACE, originally used in multiple myeloma as salvage chemotherapy regimen in patients affected by relapsed lymphomas. This regimen has advantage of the continuous infusion (CI) principle; we chose the D-PACE regimen (without Thalidomide), in which doxorubicin is administered as a continuous infusion over 72 h, in order to: 1) cytoreduce the disease in patients eligible for an allogeneic SCT 2) overcome the MDR-1-mediated resistance in tumor cells by continuous exposure at low drug concentrations 3) reduce the risk of cardiotoxicity in patients that were heavily pretreated with CHOP-like regimens. Methods. D-PACE regimen consisting of Dexametazona 40 mg days 1-4 i.v., Cyclophosphamide 400 mg/m2 CI days 1-4 i.v., Doxorubicin 10 mg/m2 CI days 1-4 i.v., Etoposide 40 mg/m2 CI days 1-4 i.v. In responding patients the regimen was planned for four cycles. Between June 2001 and July 2005 40 patients affected by relapsed or refractory lymphoma entered the study: 20 patients with Non-Hodgkin Lymphoma (NHL) (16 aggressive and 4 indolent), and 20 patients with Hodgkin disease (HD). Median age was 40 years (range 17-75). All patients were heavily pre-treated: the median number of previous chemotherapy regimens was 3 (range 1 to 6). Fifteen patients had failed a previous autologous SCT and 7 an allogeneic SCT. Results. No treatment-related deaths or serious adverse events occurred. An objective response was observed in 17 patients (43%); 6 complete remission (15%), 11 partial remission (28%). HD patients had a better OS compared to NHL patients (p=0.004), while no difference was seen in PFS between HD patients and NHL patients (p=0.46). Seventeen patients (7 NHL and 10 HD), who were in complete (30%) or partial remission (30%) after D-PACE courses, underwent an allogeneic SCT. Allotransplanted patients had a significantly better survival, if compared with the cohort of patients not eligible for allogeneic SCT (25 cases). In fact we observed an OS at 2 years of 54% and 27%, p-value (p=0.029) and a PFS at 2 years of 80% and 16% respectively (p=0.0006). Summary/Conclusion. The D-PACE regimen is an effective and very well tolerated chemotherapy, that can be used in extensively pretreated patients with relapsed or refractory lymphoma, as debulking therapy before allogeneic transplantation.
Results.

0724
LONG TERM SURVIVAL DATA OF PEDIATRIC NHL PATIENTS
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Background. Evaluating NHL treatment result data can provide insight and useful information to guide our future approach and possibly improve the care of our children. Aims. The characteristics of the patients with NHL, treated in our Department over the last 15 years are analyzed. The results are summarized in total and by the different time course of presentation and treatment schedule. Methods. From 1990 to 2005, 47 children (10 girls) were diagnosed with NHL. Mean age at diagnosis was 8.40 years (range, 0.58 to 14.5). During the 1st, 2nd and 3rd decade 14 (5), 17 (3) and 16 (4) patients were diagnosed, respectively. Based on pathology, B-NHL, T-NHL and Ki-1 (+) NHL was diagnosed in 31, 11 and 5 patients, respectively. Most common presenting sites were the mediastinum (15), the neck area (12) and the abdomen (8). For all patients, stage I, II, III, IV was found in 8, 14, 25 and 7 patients, respectively. Treatment varied through the last 15 years. The approach of the BFM protocol was applied since 1995 (BFM-NHL 90 and from 1997 the BFM-NHL 95 protocol). Irradiation was given to 5/47 patient (with B-NHL 2/5 and with T-NHL 3/5) and autologous SCT to 4 patients, all with B-NHL (1 with CNS disease, 1 with residual disease at the end of treatment and 2 at relapse). Results. Thrity eight (85) patients are alive: 55, 2 and 1 in 1st, 2nd and 3rd remission, respectively. Nine (9) patients in total have total succumbed (2 died soon after admission from other hospitals due to acute phase complications and 5 patients died during the 1st decade of our retrospective study (with T-histology and extensive disease). EFS (55 of 47 patients) is 74.4% and OS (58 of 47 patients) is 80.9%, for a median follow-up time of 6.1 years (range, 0.01 to 14.7) for all patient. For the 34 patients treated with the BFM-95 protocol since 1997, EFS and OS is 79.4% and 88.2%, respectively, for a median follow-up time of 4.8 years. Conclusions. Overall and events free survival and outcome of our patients with NHL treated during the last 15 years is standing high. Due to continuous improvement of the supportive care and understanding of the protocol philosophy while by implementing the BFM NHL treatment approach for our patients the mentioned high standing outcomes have been documented. There has been limited use of irradiation and stem cell transplantation.

0725
PREVALENCE OF HEPATITIS B IN PATIENTS WITH HODGKIN AND NON-HODGKIN'S LYMPHOMAS
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Introduction. High prevalence of hepatitis B infection has been observed in patients with lymphomas in previous studies, but it is still not clear whether there is an association between malignant lymphomas and hepatitis B virus (HBV). Aims. The aim of this study was to investigate the incidence of hepatitis B among patients with Hodgkin and non-Hodgkin lymphoma. Patients and Results. We retrospectively studied 1191 patients with lymphoma who were admitted to our hospital unit from January 1980 to December 2005. They consisted of 404 cases of Hodgkin lymphoma (HL) and 787 cases of non-Hodgkin lymphoma (NHL). Patients were tested for hepatitis B antigen (HBAg) during their first admission to the hospital. Nine out of 404 patients with HL (22.5%) and 46 out of 787 patients with NHL (5.84%) had positive HBAg. The rate of hepatitis B infection in patients with HL and NHL was higher than the general Greek population (0.9%). When compared statistically by the x2 test the prevalence in patients with HL was not significantly higher than normal persons (p<0.05), while the prevalence in patients with NHL was significantly higher than the general population (p<0.001). HBV infection is known to cause immune disorders and clonal expansion of B lymphocytes, probably contributing to lymphomagenesis. In addition the immunodeficiency that precedes and leads to chronic hepatitis B may also be a predisposing factor for the development of a malignant lymphoma. It is not known whether patients with hepatitis B have the same response rates to treatment and survival rates with the rest of the patients. Larger series of patients are needed to investigate it. Conclusions. We observed high rate of HBAg in patients with lymphomas especially NHL. Hepatitis B virus may play a role in lymphomagenesis. Further studies are required to clarify the association between HBV infection and malignant lymphomas.

0726
SAFETY AND EFFICACY OF RITUXIMAB COMBINED WITH CHEMOTHERAPY FOR LYMPHOMA DURING PREGNANCY
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Background. Management of non-Hodgkin lymphoma during pregnancy remain a difficult challenge for both patients and doctors. Treatment may allow to obtain a complete response for the mother without side effects for the fetus. Few data have been published on the safety of rituximab during pregnancy. Rituximab is a chimeric IgG1 antibody, which can cross the placenta and interact with fetal B cells. Aims. We report the case of a woman with a diffuse large B cell lymphoma during pregnancy who was treated with rituximab. Methods. A 28 year old woman was diagnosed with CD20+ diffuse large B cell lymphoma in her 18 week of pregnancy. Staging show a stage IIb with mediastinal bulky. After careful consideration and patient informed consent, the patient was treated with a combination of rituximab and chemotherapy (standard CHOP: rituximab 375 mg/m² D1, cyclophosphamide 750 mg/m² D1, doxorubicin 50 mg/m² D1, vincristine 2 mg D1, and oral prednisone 100 mg D1 to D5 given in 3-week cycles. There were no infusions reactions. During treatment, the intrauterine development was closely monitored. She received four cycles of treatment before delivery. She delivered in the 38 week of pregnancy and in very good partial remission, a 2000 g healthy child via caesarean section. Two weeks after caesarean, she was treated with two another cycles of chemotherapy with rituximab. Ten scan completed to a complete remission. The child is now 10 month old and has a completely normal growth. After a follow up of 10 months, our patient is still in complete remission. Conclusions. Little is know about the safety and efficacy of rituximab during pregnancy. To our knowledge, there are three cases treated with rituximab during the first (one patient) or second (two patients) trimester of pregnancy. Rituximab seems safe and without significant consequences for the fetus. Although B cells were extremely low at birth and during first weeks in the child, no infectious complications have been reported. Conclusions. Combination of rituximab with chemotherapy is safe and might be a valuable treatment option for pregnant women with CD20+ lymphoma. Controlled studies are necessary to confirm this data.
PET (Positron Emission Tomography) imaging uses the glucose analogue 18F-FDG as a tracer, and is an excellent method to detect small focal sites of high metabolic activity, which are frequently indicative of tumors. However, 18F-FDG uptake is not tumor specific. Various forms of inflammatory lesions and healing tissues, that have a high concentration of inflammatory cells (neutrophils, activated macrophages), also take up 18F-FDG, and are a major cause of false positive findings. 18F-FDG PET represents a major advance in both staging and restaging of Non-Hodgkin Lymphoma (NHL), however the sensitivity in the setting of restaging is lower and is associated with a significant number of false positive findings. Here we describe two cases, that illustrate the caution needed in the interpretation of PET scans in the restaging context of NHL patients. Case 1. A 57 year old male, with a solitary nodule with 10 cm of diameter, localized to the segment IV of the liver, histologically compatible with Diffuse Large B-Cell NHL (stage IE A). After treatment with R-CHOP, we observed in CT scan, a 60% reduction of the hepatic lesion dimensions. The PET/CT showed increased 18F-FDG uptake in the segment IV of the liver, compatible with the persistence of NHL. A left hepatectomy and resection of the segment IV was performed. The histological examination of the surgical specimen showed a large nodular area of tissue necrosis, surrounded by a fibrosis capsule and numerous activated macrophages, but no signs of persistent disease. One year after surgery, the patient is in complete remission. Case 2. A 53 year old male, with a solitary nodule, bulky (infra-diaphragmatic), treated with R-CHOP. After treatment, a persistent thickening of the mesenteric fat was seen in the CT scan. The PET/CT showed increased 18F-FDG uptake in multiple abdominal confluent masses, in the pre-aortic and mesenteric regions, compatible with the persistence of NHL. A laparotomy with multiple biopsies was performed. The histological examination of the surgical specimen showed large areas of fat necrosis, and no evidence of NHL. In conclusion, 18F-FDG PET is an essential tool in the management of NHL patients. However, in case of positive 18F-FDG-PET findings, histological confirmation is required, in order to exclude post treatment benign inflammatory lesions, and to avoid unnecessary treatment approaches.

Myeloma and other monoclonal gammopathies III

DIVERSE Niches Within Multiple Myeloma Bone Marrow Samples Affect Plasma Cell Enumeration and FCM Profile

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Background. The diagnosis of MM is based on the combination of clinical and laboratory criteria including bone marrow (BM) morphological assessment (percentage of plasma cells counted), often combined with flow cytometry (FCM). Aims. In this study we compared the bone marrow plasma mycosis by microscopic examination of BM aspirates, to the FCM results in samples obtained from MM patients. We also tested whether the noted discrepancy between these two methods applies only to MM, or represents a trend in other hematopoietic malignancies as well. Methods. The number of plasma cells in BM aspirates from 41 MM patients were analyzed simultaneously by morphological evaluation and FCM using the following panel of antibodies: CD38, CD45, CD138, and IgG isotype controls. Each sample was assessed independently by two qualified laboratory specialists and/or hematologist. Seven BM samples from patients with acute myeloid leukemia (AML) were compared in a similar manner. Results. In MM it was evident that FCM under-estimated the number of BM plasma cells in samples by an average of 60%, compared with conventional morphological evaluation. On the other hand in AML there was a good correlation between the morphological and FCM assessments of the blast cell population, indicating that the discrepancy observed in the MM BM samples may be related to unique characteristics of the malignant plasma cells. This discrepancy may results partially due to the fact that bone marrow aspirates contain cells associated with the lipid-enriched spicules, while flow cytometry analysis is performed on the bone marrow fluid which is depleted of these fat tissue-adhesive plasma cells. When disrupted spicules from BM BM samples were isolated (by repeated passages through 21g needle), a 40% increase in the plasma cell percentage was noted, compared with the fluid of the same BM samples. In order to determine the FCM profile of the cells in these two fractions, we isolated BM derived spicules from aspirates of MM patients, and either sheared them mechanically with repeated passages through a 21g needle, or treated them with a cocktail of three extracellular matrix (ECM) degrading enzymes (heparinase I, chondroitinase ABC and hyaluronidase), followed by mechanical shearing. Only a combination of these two methods (shearing and ECM degrading enzymes) released the highly adhesive plasma cells from the spicules. The released myeloma cells displayed a different FCM profile and in particular had a higher level of CD138 expression. Summary. We have shown a major discrepancy between the percentage of MM cells obtained by routine BM morphology and flow cytometry counts. It is possible that this discrepancy is partially attributable to the two distinct microenvironmental components occupied by MM cells in the BM sample - the lipidic spicules, and the fluid phase. MM cells located in the different niches of the BM also differ in their FCM profile. This study indicates that multiple myeloma patients contain heterogeneous populations of malignant plasma cells. These sub-populations may play distinct roles in the different biological and clinical manifestations of the disease.

Combination of Bortezomib, Melphalan, PREDNISONE and THALIDOMIDE in Advanced Myeloma: A Phase II Clinical Trial

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Background. Bortezomib (Velcade™) and Thalidomide are effective for the treatment of refractory multiple myeloma (MM). In vitro studies showed that Bortezomib can restore sensitivity to Melphalan-resistant MM cell lines [Clin. Cancer Res 2005; 11:1136-1144]. In newly diagnosed patients (pts), the addition of Thalidomide to the standard oral Melphalan/Prednisone combination significantly increased response rate and event free survival [Cancer 2005;104:1425-33]. Aims. A phase II trial was...
SERUM CONCENTRATIONS OF DICKKOPF-1 PROTEIN ARE INCREASED IN PATIENTS WITH MULTIPLE MYELOMA AND REDUCED AFTER AUTOLOGOUS STEM CELL TRANSPANTATION

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Background. Dickkopf-1 (DKK-1) protein, a soluble inhibitor of Wnt signalling, has been implicated in the pathogenesis of myeloma bone disease. DKK-1 protein was detected in plasma cells isolated from myeloma patients with bone lesions but not in normal plasma cells or in plasma cells isolated from MM patients with no lytic disease. However, DKK-1 protein was detected in myeloma patients with bone lesions but not in normal plasma cells or in plasma cells isolated from MM patients with monoclonal gammopathy of undetermined significance (MGUS) and examine possible correlations with clinical data.

Methods. Oral Melphalan was administered at 6 mg/m² on days 1-5, oral Prednisone at 60 mg/m² on days 1-5 and Thalidomide at 100 mg/day continuously. Velcade™ was administered by IV bolus on days 1, 4, 15, 22 at three dose levels: in the first cohort (10 pts) 3, in the second cohort (10 pts) at 1.3 mg/m² and in the third cohort (10 pts) at 1.6 mg/m². Each course was repeated every 35 days for a total of 6 courses. Dose Limiting Toxicity (DLT) was defined as the occurrence of any grade 3-4 non hematological toxicities, a grade 4 neutropenia > a week, or any grade 4 hematological toxicity except thrombocytopenia. Twenty pts received V-MPT as second line therapy, 16 as third line. Twenty pts received prior autologous transplant, 10 conventional chemotherapy and 9 thalidomide-based regimens. After a median of 5 courses, 20 pts (66.7%) achieved an objective response (complete response 16.7% and partial response 50%). Furthermore, 2 pts (6.7%) achieved a minimal response and 3 (10%) s; le disease. Five pts (16.7%) were refractory to treatment and experienced progressive disease. In the first cohort, 3 DLT were observed (grade 3 pneumonia, grade 3 febrile neutropenia and grade 3 vasculitis); in the second cohort, 5 DLT were observed (grade 3 Herpes Zoster infections, grade 4 thrombocytopenia and grade 4 anaemia); in the third cohort 5 DLT were observed (grade 4 thrombocytopenia, grade 3 fatigue, sensory neuropathy grade 5, grade 3 Candida esophagitis). The most common grade 1-2 toxicities were: infections, fatigue, peripheral neuropathy and constipation. After introduction of prophylaxis with acyclovir, no new HZV reactivation was observed. Among the 8 pts with baseline peripheral grade 1 neuropathy before VMP treatment, 5 worsened (one grade 3). Treatment-related neuropathy developed de novo in 4 pts (one grade 3). Conclusions. Initial results showed that VMP is a promising regimen for advanced myeloma.

OCT0730

DEVELOPMENT OF A NOD-SCID HU ANIMAL MODEL TO INVESTIGATE WALDENSTRUMS MACROGLOBULINEMIA

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Waldenström’s macroglobulinemia (WM) is a B-cell lymphoproliferative disorder characterized by predilection for bone marrow involvement and secretion of IgM paraprotein. The purpose of this study is to establish an animal model mimicking closely the disease in humans. We implanted into NOD-SCID mice human cancellous bone obtained from adults undergoing total hip arthroplasty or hemiarthroplasty. Cancellous bone was harvested in compact cores from the femoral head and was implanted in the hindlimb muscles of ten NOD-SCID mice. Mice were used when 6 to 8 weeks of age (25-30 grams). The size of the bone implant was between 16 and 22 mm³. Eight to twelve weeks after the bone implantation, 3-5×10⁸ WM cells freshly harvested from a WM patient were injected i.m. very close to the bone implant into 4 mice, and 10×10⁶ cells from the same WM patient were injected i.v. into the tail vein of 2 mice bearing human bone implants. Also, two freshly harvested bone marrow (BM) core biopsies from a patient with active WM were implanted as described. All animals had a human bone fragment from non WM individuals in the opposite hindlimb. Tumor progression was determined by monitoring human immunoglobulin M (IgM) levels in murine plasma. Immunohistoopathologic evaluation was performed on the human bone grafts, and murine tissue including the femurs, and tibia, the brain, liver, spleen, lung and kidney. One out of four mice injected i.m. into the bone fragment vicinity with WM cells showed elevated levels of human IgM indicative of the development of the disease. One out of two i.v. injected mice had elevated IgM one month following the injection of the WM cells. Both mice implanted with the bone marrow core biopsies showed a declining level of IgM directly after the implantation of the biopsy, but 3 months following the implantation IgM started increasing and reached levels above baseline. Histopathologic analy-
s is performed using antihuman reagents for expression of CD20 and IgM. Positively cells for both CD20 and IgM were found in BM core biopsies from the WM patients and the bone marrow graft opposite to the injected/implanted site. The stain was present in the cytoplasm and/or the surface of the positive cells. Murine tissue needs further histopathologic evaluation. Mice may need to be followed for more extended periods of time to fully assess the pattern of WM growth in this model. In conclusion, this SCID-hu WM model more closely resembles the human disease. It differs from the recently created WM model by Tassone et al. (2005), since the utilization of adult bone compared to fetal, along with the implantation of WM bone biopsy, allows us to study the biology of the malignant cells in their native BM microenvironment.

This study is supported by a grant from the International Waldenström Macroglobulinemia Foundation (WMF) to A.S.T. and C.E.E.

### 0732

**COMBINATION OF BORTEZOMIB AND DEXAMETHASONE FOR PATIENTS WITH AL AMYLOIDOSIS**

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**Background.** Primary systemic amyloidosis (AL) is a clonal plasma cell dyscrasia and is characterized by widespread deposition of abnormal amyloid fibrils derived from abnormal light chains, leading to multisystem organ failure. Aggressive treatment of AL amyloidosis with high-dose melphalan and autologous stem cell transplantation (HDM-ASCT) is the current choice for selected patients, while the combination of melphalan and dexamethasone is used for patients who are not eligible for HDT. Bortezomib is a proteasome inhibitor with proven activity in relapsed/refractory Multiple Myeloma, alone or in combination with dexamethasone. **Aims.** To evaluate the activity and feasibility of the combination of Bortezomib and Dexamethasone (BD) in patients with primary systemic amyloidosis. **Methods.** We treated consecutive patients with histologically proven, symptomatic AL amyloidosis who had measurable disease, defined as a serum M-spike >0.5 g/dL or urine M-spike>200 mg/24 hours or involved immunoglobulin free light chain (FLC) ≥100 mg/L and an abnormal FLC ratio. None of the patients had a history of multiple myeloma. Patients were treated with the combination of Bortezomib 1.5 mg/m2 on days 1, 4, 8 and 11, and Dexamethasone 40 mg on days 1 to 4, every 21 days, for 4-6 cycles. Dose modifications were made based on toxicity. For the assessment of hematologic and organ response we followed the recommendations of the 10th International Symposium on Amyloid and Amyloidosis (Gertz et al., Am J Hematol (2005) 79: 319-328).

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<th>Table 1. Patient characteristics.</th>
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<td>Male/Female</td>
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<tr>
<td>Age (median/range)</td>
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<tr>
<td>Light chain type kA</td>
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<tr>
<td>Bone marrow Plasma cells (median/range)</td>
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<td>Number of Major organs involved</td>
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<td>Heart involvement (number of patients)</td>
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<td>NYHA class ≥1</td>
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<td>BNP-120 pg/mL</td>
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<td>β2-microglobulin≥2.7 mg/dL</td>
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<tr>
<td>Urine protein (mg/24 hrs)</td>
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<td>Alk. Phosphatase &gt;1.5 ULN</td>
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**Results.** Over the last 6 months, 7 patients have started treatment with BD. Their characteristics are shown in table 1. Three had at least one prior therapy, one with HDM-ASCT followed by melphalan/prednisone, one was VAD and one patient with melphalan and dexamethasone. Four patients not eligible for upfront HDM-ASCT received BD as primary treatment. Among 6 evaluable patients so far, two had a complete hematological response (CR) and 3 had partial hematologic response (PR). Hematologic response was achieved 3 to 12 weeks (median 5 weeks) after the initiation of treatment. One patient who achieved CR to BD was subsequently treated with HDM-ASCT. It is too early to evaluate organ response. Toxicity was manageable; 5 patients had grade 1 orthostatic hypotension, one had grade 1 neuropathy and one patient had grade 2 fatigue, 4 had grade 1 edema, two patients had grade 1 diarrhea and two had grade 1 constipation. Dose reduction was needed only in one patient due to fatigue. None of our patients was given any other therapeutic agents. **Aims.** To assess the efficacy and toxicity profile when lenalidomide is used in combination with cyclophosphamide and dexamethasone for patients with relapsed refractory disease. **Methods.** Multiply relapsed patients were given Revlimid 25 mg po on days 1-21, dexamethasone 40 mg po days 1-4 and days 12-15, and cyclophosphamide 500 mg po days 1, 8, 15 and 21 of a 28 day cycle. **Results.** To date only 1 patient has discontinued therapy because of a failure to respond to therapy. One patient has completed 5 courses of therapy the remainder being on treatment. **Summary.** The combination of CRD is effective in heavily pretreated myeloma patients and has a manageable toxicity profile.

### 0734

**ACQUIRED ACTIVATED PROTEIN C RESISTANCE, MULTIPLE MYELOMA AND THROMBOSIS DURING THE INDUCTION PHASE OF TREATMENT**

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**Background.** Thrombosis is increasingly recognized as a common complication in patients with malignancy. Despite the common finding of VTE in patients with cancer, significance of coagulation test abnormalities predicting for deep venous thrombosis (DVT) still remain to be proven, although recently has been reported the impact of diagnosing acquired activated protein C resistance (APC) on DVT development in myeloma patients. Thalidomide has been used to treat refractory MM,
and an increased risk of thrombosis has been reported when it is employed in combination with other chemotherapeutic agents. 

Aim. The purpose of this study was to examine the association between chemotherapy, thalidomide and APC-R with DVT development in a cohort of newly diagnosed MM patients. 

Methods. One hundred and twenty newly diagnosed multiple myeloma patients were evaluated. 

Results. The frequency of response (CR, VGPR, NCR, PR) in the group of thalidomide and dexamethasone was 80% (CR 50.7%, VGPR/NCR 16.4%, PR 28.4%). From the last 50 patients, 5 presented APC-R and 3 of them developed DVT (60%). All of them received Thal/Dex as therapy. We performed an APCR re-test after disease response, showing that patients who presented any type of response developed a negative re-test. Patients with thrombosis in the VAD group developed the event shorter, median time to thrombosis in this group was 2.42 months versus 4.2 months in the thalidomide plus dexamethasone group (p = 0.005). 

A cohort of patients is being evaluated using aspirin to prevent deep vein thrombosis and MM were evaluated. Coagulation tests were performed in the last 60 patients including acquired activated protein C resistance, Leiden factor V, factor VIII, serum S and C protein. Thrombosis was documented using standard criteria and diagnostic imaging.

Time to thrombosis was defined as the period of time between multiple myeloma diagnosis and the thrombotic event. Statistical analyses were performed using SPSS version 10.0. 

Conclusions. Kaplan-Meier method. Differences in survival were assessed using the log-rank test. Results. According to ISS, 158 (31.6%) patients were classified in stage I, 169 (33.7%) in stage III and 167 (33.5%) in stage III, with median OS 69 (95% CI: 66-86), 49 (95% CI: 35-55) and 25 (95% CI: 19-27) months. According to WSS, 177 (37.4%) patients belonged to stage I, 126 (26.8%) to stage II and 167 (33.5%) to stage III, with median OS 69 (95% CI: 54-74), 43 (95% CI: 38-48) and 25 (95% CI: 19-27) months respectively. Statistically significant difference in survival was detected between all stages in both staging systems (p < 0.001). There were 42 patients with B2M < 3.5 mg/L and alb < 3.5 g/dL, who when analyzed separately, had a median OS of 40 (95% CI: 32-48) months, having no statistically significant difference with stage II patients of either staging system (p = 0.7). 

Aim. To evaluate the necessity of alb in ISS. This is due to the inclusion of all patients with B2M < 3.5 mg/L in stage I, irrespective of their alb level, while in fact, patients with B2M < 3.5 mg/L, are generally in stage II. So, alb cannot be excluded from the ISS model, since it is absolutely necessary in order to identify true low-risk patients.

0735 INTERNATIONAL STAGING SYSTEM IN MULTIPLE MYELOMA: IS ALBUMIN TRULY NECESSARY IN THE MODEL?

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Background. The prognostic significance of the combination of β-2 microglobulin (B2M) and albumin (alb) in multiple myeloma (MM) was recently confirmed by the International Myeloma Working Group in a study of 10750 patients worldwide. They subsequently employed these two factors in order to develop the International Staging System (ISS). Weber et al. in an early attempt to validate the ISS in 894 consecutive patients, questioned the adding prognostic value of alb, since they demonstrated similar results using a staging system (WSS) with B2M alone in identical cut off with ISS. Aim. To evaluate the necessity of alb employment in the model of ISS by applying ISS and WSS in a large number of previously untreated MM patients. 

Methods. Between January 1989 and January 2006, 470 consecutive patients were diagnosed with MM in our department. Ninety-two (19.6%) patients received high dose therapy followed by autologous stem cell transplantation and the rest 378 (80.4%) were treated with conventional chemotherapy. All patients were classified according to 1. ISS. Stage I: B2M < 3.5 mg/L and alb ≥ 3.5 g/dL. Stage II: neither stage I nor III. Stage III: B2M ≥ 5.5 mg/L and alb < 3.5 g/dL. Stage I: B2M < 3.5 mg/L. Stage II: 3.5 mg/L ≤ B2M < 5.5 mg/L. Stage III: B2M ≥ 5.5 mg/L. Virtually, the difference between ISS and WSS is that patients with B2M ≥ 5.5 mg/L and alb < 3.5 g/dL were classified in stage II according to ISS, while according to WSS they belong in stage I. 

We decided to analyze these patients separately in order to detect in which prognostic group they practically belong. Overall survival (OS) was estimated according to Kaplan-Meier method. 

Results. In conclusion , it is clear that in multiple myeloma thalidomide and dexamethasone is a targeted combination with other chemotherapeutic agents.

0736 MOLECULAR CHARACTERIZATION OF A PANEL OF MULTIPLE MYELOMA CELL LINES: A MODEL FOR AN INTEGRATIVE GENOMICS APPROACH


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Background. The availability of Human Myeloma cell lines (HMCLs) has significantly contributed to elucidate the molecular and biological aspects of Multiple Myeloma (MM), such as the identification of the most recurrent IGH translocations and the complex network of cytokines affecting plasma cell growth and angiogenesis. Recently, genes targeted by the chromosomal translocations, as well as the activity of novel candidate specific therapeutic agents, have been investigated in HMCLs. However, it is well known that the establishment in culture perse and the continuous passages in culture confer to the HMCLs a progresive independence from growth factors as well as the gain of multiple genetic lesions. Aim. The purpose of the present study was a detailed characterization of a panel of 23 HMCLs using a genomic integrative approach combining Fluorescence in-situ hybridization (FISH) and both gene expression and genome-wide profiling. 

Aims. The most recurrent IGH translocations were determined by FISH and RT-PCR in 23 HMCLs. Gene expression profiling (GEP) of the 23 HMCLs has been generated using Affymetrix HG-U133A high-density oligonucleotide arrays. Expression data has been analyzed with unsupervised (two-dimension-al hierarchical clustering) and supervised (SAM, Significant Analysis of Microarrays) Methods. Genome wide profiling data for 17 HMCLs has been generated on high-density SNP arrays and genome-wide analyzed to investigate copy number alterations. 

Results. In the studied panel of 23 HMCLs, 8 lines displayed the t(4;14) translocation, 4 the t(11;14), 5 the t(14;16), 2 the t(6;14), 1 the t(14;20) and 13 the t(8;14), with the consequent deregulation of the respective target genes. The unsupervised analysis performed on the gene expression data showed that only t(4;14) HMCLs could be grouped in a clearly distinguishable cluster. A subset of 6 HMCLs, 4 of which without any known IGH translocations, showed the overexpression of the members of the GAG tumor antigens, previously described as associated to unfavourable tumor progression in MM patients. Interestingly, the GEP analysis revealed that MAF expression is not strictly related to the presence of the t(4;14), since its expression was found in cell lines negative for the translocation. In the group of HMCLs overexpressing MAF or MAFB, the specific deregulation of the known MAF target genes, including CCND2 and ITGB7,
was observed. Finally, our data show that all HMCLs are characterized by a complex chromosomal pattern, the most common being the gain of chromosome arm 1q and the loss of chromosome arms 1p, 17q and 17p. Conclusions. In the present study, we extend the characterization of most of the known HMCLs, making it possible a more accurate selection as appropriate model of MM for in vitro experiments and provide insights into the characterization of novel potential genetic lesion in primary tumors.

**0737**

**MONOCLONAL GAMMOPATHY: NATURAL HISTORY STUDIED WITH A RETROSPECTIVE APPROACH**

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Background. Monoclonal gammopathy of undetermined significance (MGUS) indicates the presence of monoclonal immunoglobulin in serum without evidence of multiple myeloma (MM); Waldenström macroglobulinemia (WM), amyloidosis or other malignant lymphoproliferative disease. The prevalence of MGUS and the probability of progression to malignant lymphoproliferative disease vary between studies reflecting different populations studied and sometimes referral bias. The probability of progression from MGUS to malignant plasma cell disease is reported to be 12%, 25%, 30% at 10, 20, 30 years, respectively, in the largest series so far (Kyle et al. 2002). The size of the initial paraprotein and the non IgG type were the strongest predictors of progression. Other smaller studies have supported these findings. Although it is known that a significant proportion of cases with MGUS will progress to malignant disease, sometimes after a long benign phase, it has never been investigated in how many cases MM or WM have been preceded by MGUS. Aim: The objective of this study was to examine the natural history of monoclonal gammopathy using a retrospective approach, with a long observation period, in an attempt to estimate the proportion of MGUS that could progress to a malignant lymphoproliferative disease. Methods. Data were obtained from the Icelandic Cancer Registry for all MM and WM cases in Iceland since 1955 (1991 for WM) and compared with the Icelandic Heart Association’s (IHA) biobank registry. Frozen serum samples were found from 66 MM cases and 10 WM cases. These samples were collected between 1967 and 1999 by IHA as part of the population-based Reykjavik Study in a nonselected manner. Protein electrophoresis (PE) and immunofixation (IF) was performed on all samples from the cases and two controls for each case, matched for age, gender and sampling time. Results. Paraprotein was found with PE in 26% of the samples from cases (n=21, MM=20, WM=1) and 1.3% from controls. With IF paraprotein was found in 46% of the samples from cases (n=55, MM=52, WM=3) and 2.6% from controls. The time in years from sample collection to diagnosis was 10.14 (mean), 9 (median, range: 1-23.5) and 14.33 (mean), 13.5 (median, range: 1.8-31.4) in cases with detected paraprotein in the sample and those with no paraprotein detected, respectively. All cases diagnosed with MM or WM in the same year as the sample was collected were excluded from this analysis. The type of paraprotein detected was IgA in 33.4% of cases, IgG in 57% and IgM in 8.5%. Conclusion. This study indicates that MGUS precedes MM and WM in nearly half of the cases when analyzed with IF but only a quarter could be detected with PE. MGUS prevalence in the control subjects was in concordance with large population-based studies. The prevalence of IgA paraprotein in the MM cases with a prodromal MGUS phase was much higher than commonly reported in MGUS, reflecting the findings of other large studies that IgA MGUS has the highest risk of progression to malignant disease.

**0738**

**PHASE I STUDY OF BORTEZOMIB AND 153SM-LEXIDRONAM COMBINATION FOR REFRACTORY AND RELAPSED MULTIPLE MYELOMA**

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Background. Multiple myeloma (MM) is a highly radiosensitive B-cell malignancy and radiation therapy is an effective treatment for these patients. Recent preclinical studies have demonstrated that the bone-seeking radionuclide, Samarium Sm153 lexidronam (Sam) in combination with the proteasome inhibitor, bortezomib (Velcade [Vel]), can synergistically inhibit proliferation of myeloma cell lines in vitro and reduce MM growth in mice bearing murine MM without significant myelotoxicity. These results provide the basis for a new targeted therapeutic approach for refractory and relapsed MM patients that involve combining Vel with Sam to improve the anti-MM effects of these agents without increasing their toxicity. Aims. The primary objective of this dose escalation Phase I study is to determine safety and tolerability as well as the response rate as determined by the Blade criteria of Vel + Sam treatment for patients with relapsed or refractory MM. Methods. MM patients who had failed more than 2 prior treatments will be enrolled on this Phase 1 dose-escalation trial which involves six cohorts with three patients each. Previous treatment with Vel is allowed. Dose escalations in parallel arms are as attached. A complete treatment cycle is 8 weeks. Vel is given on days 1, 4, 8 and 11 followed by a 45-day rest period. Sam is administered only on day 3. The cycle is repeated on Day 57 if disease is stable or improved and platelets and neutrophils recover to better than or equal to Grade 1 toxicity (may be delayed for up to four weeks). Dose limiting toxicity (DLT) is defined as cycle 1 Grade 4 hematologic or Grade ≥3 non-hematologic toxicity.

**Table 1. Outcome of patient cohort.**

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<tr>
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<th>Arm 1</th>
<th>Arm 2</th>
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<tr>
<td>Sam</td>
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<tr>
<td>Vel</td>
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<tr>
<td>Cohort 1</td>
<td>0.25 mg/kg 1.0 mg/m²</td>
<td>Cohort 4</td>
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<tr>
<td>Cohort 2</td>
<td>0.5 mg/kg 1.0 mg/m²</td>
<td>Cohort 5</td>
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<td>Cohort 3</td>
<td>1.0 mg/kg 1.0 mg/m²</td>
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| Results. | Cohorts 1, 2 and 4 have been enrolled (3 patients per cohort). Two patients in Cohort 4 have shown responses, partial (n=1) and minor (n=1), and have received two cycles of treatment to date. Four patients progressed including one patient who showed a transient immunofixation complete response. Three patients in Cohort 2 have not completed their first cycle of therapy. No significant hematologic toxicities have been observed. Only one patient experienced transient fever, headache and vomiting. There have been no dose limiting toxicities to date. Conclusions. This dose escalation Phase I trial of the combination of Vel and Sam demonstrates responses in relapsed and refractory MM without significant toxicity and continues to enroll patients. Updated results from the trial will be presented at the meeting.

**0739**

**ANTI-THYMOCYTE GLOBULIN INDUCES APOPTOSIS IN MYELOMA CELLS: A BASIS FOR MYELOMA SERO-THERAPY?**

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Background. Monoclonal antibody based strategies have so far been unsuccessful in the treatment of myeloma. Polyclonal anti-thymocyte globulins (ATG) are used for in vivo T-cell depletion and have been reported to have cytotoxic activity against other cells including B-cells, dendritic cells and plasma cells. ATG is produced by immunization of rabbits or horses with thymocytes or T-lymphoblasts. Aims. We investigated the effect of ATG on myeloma cell lines and bone marrow samples from myeloma patients. We also studied the mechanisms behind ATG-induced myeloma cell death. Methods. Apoptosis was detected by flow cytometry after staining with annexin V. ZVAD-fmk was used for caspase inhibition, N-acetyl-L-cysteine (NAC) served as ROS scavenger. Results. We observed strong cytotoxic activity of ATG against myeloma cell lines and primary myeloma cells. Complement-dependent cytotoxicity (CDC) was observed in 5 of 5 myeloma cell lines (RPMY-8226, U266, KMS-12-BM, EJ and NCIB929) and bone marrow samples from 6 myeloma patients. In the absence of complement ATG still induced up to 50% apoptosis in 4 out of 5 myeloma cell lines and up to 80% apoptosis in all primary myeloma samples. Preincubation of myeloma cells with a general caspase inhibitor (ZVAD-fmk) abrogated ATG-induced apoptosis but had no effect on CDC. Preincubation with N-acetyl-L-cysteine (NAC), a ROS scavenger, blocked ATG-induced CDC but had no effect on ATG-induced apoptosis. Absorption of ATG on primary T-cells completely removed anti-myeloma cytotoxicity. Conclusions. ATG induces complement mediated ROS-dependent lysis and caspase-dependent apoptosis in myeloma cells. This effect is probably due to a direct effect on the myeloma cell.
to antibodies against epitopes also expressed by peripheral blood T-cells and not specific for myeloma cells or thymocytes and lymphoblasts used for the production of ATG.

**0740**

**IMMUNOGLOBULIN-LIKE TRANSCRIPT 2 IS NOT DIFFERENTIALLY EXPRESSED IN MGUS AND MYELOMA, BUT APPEARS TO BE DOWNREGULATED AT AN EARLIER STAGE OF PLASMA CELL DISEASE**

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**Background.** Immunoglobulin-like transcript 2 (ILT2) belongs to the Ig superfamily and has homology to the killer cell inhibitory receptors (KIRs). Like KIRs, ILT2 delivers an inhibitory signal upon interaction with MHC class I ligands. It is expressed on natural killer (NK) cells, which lyse transformed or virally infected cells that have lost or downregulated expression of self MHC class I molecules. ILT2 is also known to be expressed on monocytes, macrophages, dendritic cells, and (naive) B lymphocytes. A differential expression of ILT2 was described for monoclonal gammapathy of undetermined significance (MGUS) and myeloma (Davies et al., 2006). Seven MGUS patients and 24 newly diagnosed myeloma patients were studied by gene expression profiling using Affymetrix GeneChip arrays. ILT2 was downregulated 8.26 fold in myeloma as compared to MGUS, being the most differentially expressed gene between these two subsets. However, as RNA from CD138+ cells was used in this analysis, a varying percentage of normal, non-malignant plasma cells will impact on the results, especially in MGUS cases. We aimed to define whether ILT2 expression in different plasma cell subsets (normal vs. malignant) in MGUS and myeloma and the eventual prognostic impact of a differential expression level. Methods. ILT2 expression was measured by flow cytometry using a PE-conjugated antibody (clone HP-F1, Beckman Coulter, Inc.) in a series of 30 MGUS patients and 91 myeloma patients. Phenotypically normal and malignant plasma cells were defined by differential expression of markers CD38, CD45, CD19, and CD56. Expression levels are given as mean fluorescence intensity (MFI) after correction for background staining. Results. ILT2 is not differentially expressed between MGUS (MFI median 112.0, range 13-145-274.42) and myeloma cells (MFI median 96.64, range 0.4-454.48). In contrast, MGUS/myeloma cells showed a lower expression of ILT2 as compared to phenotypically normal plasma cells in the majority of samples. An intranidividual comparison revealed a decrease in MFI in 70% of cases by a median of 63.3%, while in 30% of cases, there was an apparent upregulation of ILT2 in malignant cells (median increase in MFI of 80%). For myeloma, the variable level of ILT2 expression was confirmed by quantitative real time PCR in 26 cases. ILT2 levels did not vary with state of disease (newly diagnosed versus progressive disease). Also, we found no correlation of ILT2 expression with clinical parameters or prognosis in our series of myeloma patients, although, interestingly, 5 myeloma cell lines were completely ILT2 negative. Summary/Conclusions. ILT2 seems to be downregulated in the majority of cases at an early stage of plasma cell disease, i.e. upon transformation from a normal plasma cell to the MGUS/myeloma stage. The level of residual ILT2 expression in malignant plasma cells is neither correlated to the state of disease (MGUS versus newly diagnosed myeloma versus advanced disease), nor to prognosis of myeloma patients or other clinical parameters.

**0741**

**A NEW MODEL PREDICTING AT LEAST A VERY GOOD PARTIAL RESPONSE IN PATIENTS WITH MULTIPLE MYELOMA (MM) AFTER 2 CYCLES OF BORTezOMIB-BASED THERAPY**

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**Background.** We recently reported that Velcade, Doxil, and Dexamethasone (VDD) is very active in both newly diagnosed and relapsed MM producing overall response rate up to 94% and complete and near complete response rate (CR/nCR) up to 33%. Despite these excellent response rates, the majority of patients do not achieve ≥90% reduction of disease (or ≥VGPR) which is considered a predictor of a longer remission and survival. Several recent studies showed that modification of therapy in treatment in poor responders can improve quality of response. However, there are no established models to make early prediction of failure to achieve ≥VGPR. Aims. Using VDD as a model of Velcade-based therapy, we analyzed whether combination of normalization of free light chain (FLC) ratio and reduction of serum M-protein can be used as an early predictor of ≥VGPR response in MM. Methods. Thirty-six patients who were enrolled on IRB approved phase II trials with VDD in newly diagnosed and relapsed MM were eligible for analysis. Ultimate responses were assigned using the EBMT criteria after 6 cycles or if after 2 cycles of chemotherapy the patient exhibited at least VGPR. Patients received a score of 1 if they had either normalization of FLC ratio (provided there was a reduction in involved FLC by ≥90% from the baseline at the end of treatment) or reduction in serum monoclonal protein by ≥90% after 2 cycles. If both criteria were met a score of 2 was assigned. If neither were met a score of 0 was given. The Fisher’s exact test was used to compare the score in patients exhibiting a ≥ VGPR to those with ≤ VGPR. Results. Of the 22 evaluable patients with VDD in relapsed disease, 7 exhibited a ≥ VGPR, with 15 ≤VGPR. Of the 14 evaluable patients with VDD as first line, 7 demonstrated a ≥ VGPR, and 7 ≤ VGPR. All patients with ≥ VGPR except for one in relapsed VDD protocol, had a score of 1 or 2 compared to those with ≤ VGPR, who all had a score of 0 (p=0.0001). Summary/Conclusions. In both relapsed and first line therapy, a normalization of FLC ratio or reduction of serum monoclonal protein by ≥90% after 2 cycles of chemotherapy accurately predicts at least VGPR response to chemotherapy. In future trials with Velcade-based regimens, early modification of therapy could be planned if a patient does not demonstrate a normalization of FLC or ≥90% reduction of serum monoclonal protein after the initial 2 cycles.

**0742**

**RARE OCCURRENCE OF T(4;14) AND P53 DELETION BUT HIGH INCIDENCE OF OTHER MYELOMA HIGH-RISK FEATURES (+1q, +q0, AND 13q-) IN MGUS ANALYSIS USING FISH AND DNA PROBES FOR THE DETECTION OF GENOMIC ABNORMALITIES INVOLVING 10 CHROMOSOMAL LOCIS**

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**Background.** The biology of the transition of monoclonal gammapathy of undetermined significance (MGUS) to multiple myeloma (MM) is poorly understood but one concept holds that this process is closely linked to the accumulation of clonal aberrations in the neoplastic cell. In MM, several chromosomal abnormalities (13q-, 17p-, +q0, t(4;14) and amplification of CKSB1 at 1q21.2) are of prognostic relevance as they are associated with shorter survival. Methods. So far, bone marrow specimens from 48 patients diagnosed with MGUS at our institution were analyzed by FISH and a DNA probe set originally designed for the evaluation of MM. The probe set comprises probes mapping to chromosome bands 1p22, 1q21.2, 6q2, 18p11, 9q34, 11q25, 13q14, 17p15, 22q11.1, and 14q32 (including probes for the detection of t(11;14) and t(4;14)). Purification of PC by immunomagnetic separation (CD138) was performed in 59 of 45 cases. Results. The most frequent chromosomal imbalances in the entire cohort were: +q0 (13/44-29%), t(11;14) (8/28-28%), +1q (10/48-21%), 13q- (10/48-21%), and +1q (9/46-19%). No p53 deletion was detectable in 48 patients. Chromosomal extra copies were significantly more prevalent in patients lacking an IgH translocation (p=0.047). Conclusion. Detection of clonal abnormalities as that of +q0 by FISH is highly prevalent in MM - are frequently found in MGUS while t(4;14) seems to be rare. No p53 deletion was found in the present series. Chromosomal extra copies were significantly more prevalent in patients lacking a 14q32 translocation.

**0743**

**SCREENING OF JAK2 V617F MUTATION IN MULTIPLE MYELOMA**

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**Background.** JAKs tyrosine kinases are important mediator of cellular signals between cytokines, receptors and effector proteins. They have 7 structural domains 'JAK homology regions' (JH1-JH7) in particular JH1 and JH2: JH1 has kinase activity, while JH2 has a negative regulatory function on JH1. Recently a somatic mutation in exon 12 of JAK2 has been described in myeloproliferative diseases Philadelphia Chromosome negative as PV, ET and IM and more recently this mutation has been investigated also in AML, MDS, aCML (BCR-ABL negative), ALL and CLL. JAK2 mutation was identified in a subset of CMMML/ aCML, and...
OZONE THERAPY IN THE TREATMENT OF OSTEONECROSIS OF THE JAWS IN MULTIPLE MYELOMA PATIENTS

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Background. Bisphosphonate (Pamidronate and/or Zoledronate) therapy is commonly prescribed for the prevention and cure of pathologic skeletal events in Multiple Myeloma (MM) patients. However, these drugs lack specificity for MM and their effects on bone resorption are due to their osteoclastic inhibition results in reduction of bone resorption. Since 2003 a possible association between bisphosphonate use and the appearance of osteonecrosis of the jaws, especially in long-treated patients undergoing invasive oral procedures, has been reported. Histologic examinations show these lesions to be the result of an avascular necrosis of the bone, due, in first iotophecy, to bisphosphonates osteoclastic and angiogenic inhibition, which impairs healing and exposes to infections by oral bacteria. Aims. The limited benefits recorded in patients suffering from this severe complication with the treatment options so far utilized, i.e. antibiotics with or without surgery and hyperbaric oxygen therapy, prompted us to explore the use of ozone therapy, whose antimicrobial action and neoangiogenetic properties have been shown capable of arresting the progression of dental lesions. Methods. Since 1998 up today, in our Institute we treated 311 Multiple Myeloma patients with Pamidronate 90 mg i.v. and/or Zoledronate 4 mg i.v. monthly. Twenty-two (7%) patients referred toothache, impaired healing after teeth extractions, dental abscesses and bone exposure, bisphosphonates were withdrawn and antibiotics administered. Considering the limited benefits in this subset of patients with the standard therapy we decided to follow a 15 day treatment protocol including antibiotics (amoxicillin-clavulanic acid 2 g/daily plus metronidazole 1 g/daily), surgery (from simple debride to bone resection) and ozone therapy (administered previous, during and after surgery). Response: Among the 22 patients with dental abscesses or jawbone exposure, 12 patients are evaluable for response because they completed the program. Ten were symptomatic MM treated with chemotherapy and 2 smoldering myelomas with a median age of 67 years (range 58-79). Eight patients were IgG, 3 IgA and 1 light chain. Seven patients had received Zoledronate and 5 Pamidronate followed by Zoledronate for a median time of 19 months (range 6-63). One patient had a wide bone exposition with oro-sinusal fistula, 10 had difficult healing after teeth extractions or oral cleaning and 1 developed ONJ spontaneously. Aims using this protocol and biotherapy that were evaluated by questionnaires administered before and after the treatment. Nine patients (75%) have witnessed complete resolution of the problem with a total reimplantesimalization of the lesions and 4 (25%) an objective improvement. Conclusions. These results demonstrate that the association of ozone therapy with antibiotics and surgery is an effective treatment for avascular necrosis of the bone.

EFFECTS AND MOLECULAR MECHANISM OF LENALIDOMIDE ON FGF SIGNALING IN ENDOTHelial CELLS AND FGFFR3+ MULTIPLE MYELOMA CELL LINES


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Background. Lenalidomide (Revlimid®) has recently been approved for the treatment of a subset of myelodysplastic syndromes, and is being evaluated as treatment for a broad range of other hematologic and oncology conditions, including multiple myeloma (MM), chronic lymphocytic leukemia and solid tumor cancers. Data from Phase II and Phase III studies of lenalidomide plus dexamethasone in MM patients show significant benefits of combination therapy versus dexamethasone alone, in terms of median time to disease progression and overall survival. A subset of MM patients (10-20%) are known to have a t(4;14) translocation that results in the overexpression of oncogenes FGFR3 and MMSET/WHSC1. FGFR3 is not normally expressed in B cells but is overexpressed and sometimes has a constitutively activating mutation in multiple myeloma cells with t(4;14). FGFR3 is an oncogenic tyrosine receptor kinase that is activated by the pro-angiogenic growth factors aFGF and bFGF. FGFR3 signals activate the MAPK pathway via Shc and GRb2 scaffolding complexes in FGFR3+ MM cells, while FGF signals activate the Akt pathway in ECs. We hypothesized that inhibition of FGFR3 signaling may be one of the mechanisms of lenalidomide action. Aims. The present study examines the effect of lenalidomide on FGF-induced signals in endothelial cells and t(4;14) MM cells. Methods. EC migration assay. HUVECs (5x10⁴ cells/insert) were assayed for migration in response to bFGF (0.1 ng/ml) using the BD Becton Dickinson Angiogenesis Assay. IC50s were calculated by nonlinear regression analyses with GraphPad Prism. Immunoblot. Cells were treated with DMSO or lenalidomide for 72 hours and assayed by 3H-thymidine incorporation.
TUMOR ANGIOGENESIS AND SENSITIVITY TO THE IL-6 IN MULTIPLE MYELOMA: EXPRESSION OF THE MICROVASCULE DENSITY AND GP-130 INTERLEUKIN-6 TRANDUCER WITHIN THE BONE MARROW COMPARTMENT

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The functional interplay between the myeloma cells and the surrounding microenvironment within the bone marrow (BM) includes increased activity of endothelial cells resulting in neovascularisation, and enhanced sensitivity to the IL-6 as a main growth factor in multiple myeloma (MM). This cytokine, as a member of gp130 family activity of endothelial cells resulting in neovascularisation, and enhanced racion of the chr plasm immunoglobulin staining (cIg-FISH) were used to detect the aber-
ration of the chromosome 13 (cut off levels 9%, 20%, 80%). We have correlated standard prognostic factors (MIG, LDH, β2M, Hb, platelet count, albumin), EFS, and OS with the occurrence of the aberration of chromosome 13 using three variants of cut off levels (9%, 20%, 80%). Higher MIG and lower albumin concentrations and platelet counts (for cut off level 80%) were detected in patients with aberration of chromosome 13 (cut off levels 9%, 20%). This analysis will be extended for all centres of CMG 2002.

CORRELATION BETWEEN THE CYTOGENETIC FINDINGS AND THE PROGNOSTIC FACTORS IN THE GROUP OF PATIENTS FROM THE CMG 2002 CLINICAL STUDY

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Background. Cyto genetic abnormalities in multiple myeloma (MM) are one of the most important independent prognostic factors. Aims. To determine the correlation between the aberration of the chromosome 13 (detected by molecular cytogenetic methods) and the prognostic factors in the pilot group of patients from the CMG 2002 clinical study (only data from one clinical centre covers 1/4 of patients) using three various cut off levels (9%, 20%, 80%). Methods. Interphase fluorescence in situ hybridization (I-FISH) and fluorescence in situ hybridization and clg-FISH were used to detect the aber-
ration of the chromosome 13. Cyto genetic abnormalities were found in 65 newly diagnosed patients with MM, the median of follow up was 22.8 month. Results. The aberration was found in 40% (26/65) patients (cut off levels 9%, 20%) and in 21.5% (14/65) patients (cut off level 80%). We have correlated standard prognostic factors (MIG, LDH, β2M, Hb, platelet count, albumin), event free survival (EFS), and overall sur-
vival (OS) with the occurrence of the aberration of chromosome 13 detected by I-FISH on bone marrow slides and clg-FISH. Higher MIG and lower albumin concentrations and platelet counts were detected in patients with aberration of chromosome 13 (cut off levels 9%, 20%), similar results were obtained for cut off level 80%. No prognostic sig-
nificance was found between aberration of chromosome 13 and the worst prognostic feature (EFS shorter than one year) in all cut off levels for aberration of chromosome 13. Summary/Conclusion. We have analysed the data from homogenous group of patients undergoing autologous transplantation in the CMG trial of Czech Myeloma Group. We have correlated standard prognostic factors (MIG, LDH, β2M, Hb, platelet count, albumin), EFS, and OS with the occurrence of the aberration of chromosome 13 using three variants of cut off levels (9%, 20%, 80%). Higher MIG and lower albumin concentrations and platelet counts (for cut off level 80%) were detected in patients with aberration of chromosome 13 (cut off levels 9%, 20%). This analysis will be extended for all centres of CMG 2002.

LOW-DOSE THALIDOMIDE AS MAINTENANCE THERAPY FOLLOWING SINGLE OR TANDEM AUTOTRANSPANT IN ADVANCED MULTIPLE MYELOMA IMPROVES OVERALL RESPONSE WITH MILD TOXICITY

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Background. Thalidomide has been introduced few years ago in the treatment of MM. At present is part of many clinical trials, especially as front line therapy in combination with desamethasone or chemothera-
py. Although the activity of Thalidomide as monotherapy is widely accepted in relapsed or refractory MM, its role as maintenance therapy following autotransplant is still under investigation. The drug is effective but the toxicity, i.e the DVT, remains one of the main reasons of concern for many investigators, so that the schedule, dose and anti-thrombo-
otic prophylaxis are still matter of debate. Methods. In 1999 we started a trial with conventional chemotherapy (3 cycles of VAD), followed by high-dose cyclophosphamide (7 g/m 2 i.v.) and peripheral stem cells (PBSC) harvest, followed by single or tandem autotransplant with mel-
phalan (200 mg/m 2 i.v.), in patients affected by advanced MM (stage II-III Salmon-Durie). Thalidomide 100 mg a day was then given as main-
tenance to all patients regardless the type of response, and discontinued at the time of relapse or progression, or for toxicity. No anti-thrombot-
ic prophylaxis has been administered. Patient characteristics. Between Jan-
uary 1999 and June 2005, 75 consecutive MM patients were enrolled. Seventy patients, median age 55 (range 46-66 years), M/F 43/27, are val-
uable. All these patients completed chemotherapy without major prob-
lems, and no toxic deaths occurred: 10/70 patients were in complete remission (CR) at the time of PBSC transplant, 34/70 reached CR after transplant (60/70 cases underwent tandem transplant), so that after chemotherapy 44/70 (62%) were in CR, defined as bone marrow plasma cytosis below 5% and absence of serum and urine paraprotein. Thalidomide was started when possible within 6 months following transplant: 21/70 patients could not be treated because of different rea-
sions: progression of disease (6 cases), thrombosis, infections, psy-
chological problems (3), performance status <70% (2), neurological problems (2), refusal (1). Three cases were followed in other Institutions. Only 4 patients discontinued the drug in few weeks because of mild neurological toxicity (WHO < 2). The remaining 49 patients (70%) continued the drug until relapse or progression, for a median time of 24 months after transplant. Results. The CR rate after PBSC transplant was 69% in the group treated with thalidomide and 52% in the remaining patients. With a median follow-
up of 38 months we compared the number of relapses/progressions, the time to relapse/progression, the disease free survival (DFS) and the over-
all survival (OS) in the two groups of patients. Toxicity. Most patients reported peripheral neuropathy, somnolence and constipation: when severe, a temporary adjustment of drug dose was able to control these symptoms. Despite the absence of anti-thrombotic prophylaxis, no DVT were observed. Conclusions. Low-dose Thalidomide following single or tandem autotransplant appears to be a safe and feasible maintenance treatment improving overall response rate without severe side effects. No anti-thrombotic prophylaxis is needed.

Table 1. In results, after in the two groups of patients.

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0749 PEGYLATED LIPOSOMAL DOXORUBICIN, MELPHALAN AND PREDNISONE THERAPY FOR ELDERLY PATIENTS WITH MULTIPLE MYELOMA

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Background. Melphalan & Prednisone (MP) is considered as the standard therapy for Multiple Myeloma (MM) patients not eligible for high dose therapy, but the addition of new drugs could result in better results. Aims. Here we report the results of a phase I-II study to evaluate the feasibility and efficacy of the association of PLD to the conventional MP regimen during the first 6 cycles of the front-line therapy for untreated MM patients older than 70.

Patients and Methods. Thirty patients were included in the study with a median age of 77 years (71-84) and a M/F ratio of 17/13 in a phase I/II study to determine the best dose of PLD and the response rate. Results. The phase I of the study demonstrated that the maximum tolerable dose of PLD in this setting was 30 mg/m2, so it was the final dose evaluated in the study. 29 patients were valuable for response, which was: complete in 4 (14%), partial in 15 (52%), minor/no changes in 7 (24%) and progressive in 3 (10%). The median progression free survival (PFS) was 24 months. The median overall survival (OS) has not been reached yet, with a 3-year probability for OS and PFS of 52% and 37%, respectively. Hematological toxicity was frequent but usually weak/moderate (grades 1 & 2 of the WHO scale) and it was resolved only with dose delays. Infection was a relatively frequent event (50% of patients), but only in 4 cases it was of grade 3. No cases of palmar-plantar erythrodysesthesia were observed. Conclusions. Elderly MM patients can benefit from other more intensive therapeutic alternatives than MP as the addition of pegylated liposomal doxorubicin to this conventional regimen.

0750 INCREASED INHIBITORY, CD158A, RECEPTOR EXPRESSION ON CD16+NK CELLS AND IMPAIRED NK CELL CYTOTOXICITY IN ADVANCED MYELOMA PATIENTS

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Background. The inability of the immune system to recognize and kill malignant plasma cells in patients with multiple myeloma (MM) has been attributed in part to the ineffective activation of natural killer (NK) cells. The activity of NK cells is regulated by opposing, activating and inhibitory, receptors and their balance, as well as the influence of cytokines, determines NK cell cytotoxicity. The aim of this study was to evaluate NK cell activity in the light of the expression of novel NK cell activating and inhibitory receptors in myeloma patients.

Methods. In this study in 20 MM patients in clinical stage III and IV, prior to therapy, and in 15 controls NK cell activity, percent of innate cell subsets, expression of activating (CD161) and inhibitory (CD158a, CD158b) receptors on freshly isolated PBL and CD16+NK cells were evaluated using 51-chromium release assay and direct immunofluorescence by Flow cytometry. Results. We show significant impairment of NK cell activity without any change in the percent of innate immunity subsets (CD16+NK, NKT and CTLγδ). There is a significant increase in the CD16dim NK cell subset in PBL in MM patients compared to controls. There is no decrease in CD161 activating receptor (MFI of CD161 on CD16bright is significantly higher), or increase in CD158b inhibitory receptor, expression on fresh PBL or CD16+NK cells, while, there was a significant increase in the inhibitory, CD158a, receptor expression on CD16+NK cells in MM patients. Conclusion. We give novel results for advanced multiple myeloma patients that show that an increase in the immature CD16dim NK cell subset and an increase in the expression of KIR, CD158a inhibitory receptor, on CD16+NK cells has an adverse effect and is associated with impaired NK cell cytotoxicity. Aside from this, these findings may have implications in developing therapeutic approaches in multiple myeloma which use recombinant NK receptor ligands that aid in targeting NK cells to tumor cells.
Myeloma and other monoclonal gammopathies IV

0751
THE RISK OF THROMBOCYTOPENIA AND NEUROPATHY AFTER BORTEZOMIB THERAPY DEPENDS ON THE BASELINE PLATELET COUNTS AND PREVIOUS NEUROPATHY RESULTS OF CHECZ MYELOMA GROUP

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Thrombocytopenia and neuropathy are the most frequent and serious complications of bortezomib therapy. However, previous data suggested that the risk of these adverse events depends on the baseline involvement associated mostly with previous therapy. Bortezomib as monotherapy was given to 82 consecutive patients with refractory/refractory multiple myeloma in 5 centers in Czech republic. Thrombocytopenia and neuropathy were, of course, the most frequent complications, observed in 65.9% and 52.4% of patients, respectively. In 68 patients baseline platelet counts were within normal range, in 38 of them thrombocytopenia developed during the course of therapy. Nineteen patients had mild thrombocytopenia before the start of bortezomib treatment. The risk of thrombocytopenia was 60.5% (grade 3/4 thrombocytopenia 30.2%) and 59.2% (grade normal baseline values and 84.2% (grade 3/4 63.2%) in patients with previous abnormality. Similar relationship was observed in the case of neuropathy. The risk of neuropathy was 45.2% in patients without any signs of neurological involvement before the start of therapy compared with 75% risk in the patients with grade 1 of any type of neuropathy in the history. Grade 3/4 neuropathy occurred in 1.6% of patients in the first group and in 25% in the second one. Conclusions. Thrombocytopenia and, especially, neuropathy could lead to dose adjustment and/or premature termination of bortezomib therapy in myeloma patients. The association between the risk of these complications and the baseline involvement supports the statement that it would be advantageous to start treatment with bortezomib earlier in the course of disease.

0752
BORTEZOMIB (VELCADE) IN COMBINATION WITH LIPOSOMAL DOXORUBICIN (DOXIL) AND THALIDOMIDE IS AN ACTIVE SALVAGE REGIMEN IN PATIENTS WITH RELAPSE OR REFRACTORY MULTIPLE MYELOMA: FINAL RESULTS OF A PHASE II STUDY

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Background. Tumor microenvironment (ME) plays an important role in MM. It is associated with disease progression, metastasis, and resistance to therapy. Therefore, targeting the ME and the tumor cell simultaneously may be an effective way to overcome resistance in pts with rel/ref MM. Aims. Osolowski et al. reported improved anti-tumor responses when bortezomib (V) was combined with doxil (D) in pts with hematologic malignancies. We investigated clinically, this approach i.e., targeting the MM cell as well as its ME, using a combination of V and D and low-dose thalidomide (T) as salvage therapy for pts with rel/ref MM. Here we report the final results of this phase II study. Methods. All pts with rel/ref disease were eligible for this study. V was given at 1.3mg/m2 (D1,4,15,18) and D at 20mg/m2 (D1,15) every 4 weeks with daily T (200mg). VTE was noted. No significant non-hematologic Gr. III/IV toxicity were seen. Despite prior exposure to anthracyclines, we did not noted any cardiotoxicity with D. Conclusions. Pt with rel/ref MM usually have aggressive disease with paucity of effective regimens. VDT is a highly active salvage regimen that demonstrates high response rates including CR and acceptable toxicity in patients with relapsed/refractory multiple myeloma. Responses were noted despite prior failure of steroids, T, A and even V. VTE does not appear to be a problem with this low dose coumadin prophylaxis. Final results of this phase II study will be presented at the annual EHA meeting.

0753
FLOW CYTOMETRIC IMMUNOPHENOTYPIC ANALYSIS OF 25 CASES OF PLASMA CELL LEUKEMIA

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The aim of the study was to determine expression of adhesion molecules CD11a (LFA-1), CD18 (LFA-1γ), CD11b, CD29, CD49d, CD44 (H-CAM), CD54 ((ICAM-1), CD56 (N-CAM) and CD117 (c-kit) on peripheral blood (PB) and bone marrow (BM) lymphoid cells in 25 plasma cell leukemia patients (PCL), at diagnosis and in the control group of 10 healthy subjects. Immunophenotyping was performed on freshly collected blood and bone marrow samples by means of flow cytometry. Plasma cells were identified as showing high-density expression of CD38 and CD138 (syndecan-1). Results. of analysis were presented both as relative and absolute (omitted in abstract) values of numbers of cells with antigen expression and as relative fluorescence indices (RFIs) of studied antigens. Statistical analysis was performed using Wilcoxon’s test. All below presented differences are statistically significant. The results revealed in PCL patients a significantly higher relative and absolute number of CD54+ cells in (brackets: means±SD of PCL pts vs control) both in BM (63±29% vs 13±5%) and PB (49±25% vs 8±3%) as well as that of CD38+ cells both in BM (84±12% vs 54±11%) and PB (74±11% vs 52±7%). In turn, PCL patients showed a decreased relative number of BM: CD11a+cells (40±28% vs 73±10%), CD18+cells (47±25% vs 88±7%), CD11a+CD18+cells (42±27% vs 72±10%), CD44+cells (71±26% vs 95±4%), CD11b+cells (17±19% vs 5±10%) and PB: CD11a+cells (58±28% vs 96±3%), CD18+cells (58±29% vs 99±0,2%), CD11a+CD18+cells (58±29% vs 96±3%), CD44+cells (86±15% vs 98±0,9%). In BM of PCL patients compared with the control there were found decreased RFIs of CD18 (15,5±1,3 vs 16,6±0,7) and CD29 (8,6±1,4 vs 10,4±0,8) and increased RFIs of CD34 (16,9±2,5 vs 15,0±0,5) and CD11a (18,4±1,5 vs 14,7±0,9). In PB of PCL patients RFIs of CD29 (10±4,1) was lower than this in control (11,6±0,9) while RFIs of CD38 (16,9±3,0 vs 14,8±1,3), CD54 (16,1±2,8 vs 12±3,0), CD11a (20,4±1,8 vs 18,3±0,8) were higher. BM leukemic cells with strong CD38 expression and CD138 expression showed antigen coexpression in following number of cases: CD54 in 16/19 (82% tested), CD29 in 12/12 (100%), CD49 in 9/9 (100%), CD44 in 9/11 (82%), CD11a in 3/20 (15%), CD11b in 3/12 (25%), CD18 in 2/17 (11%), CD56 in 13/25 (56%), CD117 in 5/13 (38%) and CD19 in 0/13 (0%) tested cases. PB leukemic cells showed coexpression of CD34 in 17/19 (89%), CD29 in 12/13 (92%), CD49 in 11/11 (100%) and CD4 in 9/10 (90%), CD11a in 9/10 (90%), CD11b in 9/12 (75%) and CD56 in 11/17 (64%), CD56 in 13/22 (59%), CD117 in 5/13 (36%), CD19 in 0/16 (0%) of tested cases. Conclusions. Immunophenotype of leukemic plasma cells characterizes mainly increased expression of CD38, CD54 and CD138 also expression of CD29, CD49d, CD44 and disturbed expression of CD11a and CD11b. In one half cases tumor cells show expression of CD56 and CD117.

0754
INORGANIC POLYPHOSPHATE IS PRESENT AND INDUCES APOPTOSIS SPECIFICALLY IN HUMAN PLASMA CELLS

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Backgrounds. Inorganic polyphosphate (polyP), a ubiquitous phosphate polymer with ATP-like bonds, participates in a variety of functions including blood coagulation and cell proliferation. Recently, we have reported that human platelets have massive quantities of polyP accumulated in dense acid granules known as acidocalcisomes, in a similar way to that occurring in unicellular microorganisms (Ruiz et al. J Biol Chem 2004:279:44250). Aims. We have investigated here the presence of polyP in human plasma cells (PC), responsible for the production and maintenance of antibodies in response to antigens. We also study the effects of extracellular polyP in the biology of the human PC. Methods. We measured levels of polyP in the U266 and IM9 myeloma cell lines by a
CIP/KIP family of cyclin dependent kinase inhibitors that negatively as one of the most frequent molecular changes in haematological malignancies, DNA methylation, leading to silencing of regulatory genes, has emerged. We have also used human male genomic DNA unmodified on 2% agarose gel. Bone marrow DNA from healthy donors served as negative control. We have detected an unexpected inhibition of Ig secretion and a stimulation of apoptosis. Polyp takes over the role of cell cycle deregulation. Ablation of DNA methylation, leading to silencing of regulatory genes, has emerged.

Methods

1. 12 consecutive MM patients (9 male, 3 female, age range 50-83, median 59) and 2 consecutive WM patients (1 male and 1 female, age 75 and 47 years).

2. Analysed group of 96 patients (30 patients with MGUS and 66 patients with MM) was stratified into 2 groups according to Mann-Whitney. The relation of PINP and VEGF to any of the evaluated prognostic factors was found to be significant. The relation of serum levels of sIL-6R (< > 100 IU/l) to β-2-microglobulin and C-reactive protein (CRP) was found to be significant. The relation of serum levels of sIL-6R (< > 100 IU/l) to β-2-microglobulin and C-reactive protein (CRP) was found to be significant.
evaluation of serum levels of analysed parameters were used following Methods. radioenzymatic assay (thymidinekinase), radioimmunoanalysis (β-2-microglobulin, ICTP, PINP), method of enzymoimmunoassay (sIL-6R, sCAM-1, sICAM-1, sOPG and sRANKL) and the technique of quantitative sandwich enzymatic immunoassay (sHGF, sVEGF, bFGF, syndecan-1/CD138 and sFas). Statistical analysis was carried out using Pearson’s χ² test and nonparametric U test according to Mann-Whitney (p<0.05). Results. Statistically significant differences were found out between MGUS and MM in case of comparison of serum levels of sIL-6R (p<0.02), ICTP (p=0.001), sHGF (p<0.001) and syndecan-1/CD138 (p<0.001), whereas in case of sVCAM-1, sICAM-1, PINP, sOPG, sVEGF and sFas there were no statistically significant differ- ences. Within the analysis of the frequency of the occurrence of abnor- mal values in the MM and MGUS group there were significant differ- ences not only in the case of standard parameters such as β-2-microglobul- ulin, thymidinekinase creatinine and albumin, but also in the case of sIL-6R, ICTP, sHGF, and syndecan-1, however not in the case of compar- ison of the values of sVCAM-1, sICAM-1, PINP, sOPG, sVEGF and sFas. Measurement of serum levels of sRANKL and soluble form of bFGF was of no avail due to very low values of these parameters. Conclusion: The analysis of the 10 parameters, that are altogether very close related to the biological properties of clonal plasma cells or to the changes of bone marrow microenvironment revealed from the point of view the contribution for the combination of MGUS from MM that the only statistically significant parameters were only the serum levels of sIL-6R, ICTP, sHGF and synde- can-1 (sCD138), i.e. the parameters with certified significance for the MM prognosis evaluation.

**0759**

**VEGF EXPRESSION AND MICROVESSEL DENSITY IN PATIENTS WITH MULTIPLE MYELOMA: CLINICAL AND PROGNOSTIC SIGNIFICANCE**

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**Background.** Angiogenesis or new vessel formation is an essential component in the growth and progression of solid malignancy. However, conflicting data are reported on clinical significance of VEGF deregulation and microvessel density (MVD) in multiple myeloma (MM). Aim: To evaluate the incidence of VEGF expression and grade of MVD, and to correlate these findings with pathological and clinical features of newly diagnosed myeloma patients.

**Patients and methods.** We analyzed bone marrow biopsy specimens obtained from 59 patients with MM. The clinical staging was done according to the Durie and Salmon classification (four patients had disease stage I, 15 patients stage II and 39 patients stage III). Expression of VEGF and MVD were analyzed using standard immunohistochemical analysis of B5-fixed and routinely processed, paraffin-embedded bone marrow specimens with antibodies against VEGF and CD34, respectively. Immunohistochemical analysis was performed using standard biotin–avidin–peroxidase technique. MVD was evaluated by counting number of microvessels in three hot spots x400, according to the method of Weidner et al. VEGF immunoreactivity was estimated on the basis of intensity and percentage of positive plasma cells. Results. VEGF was expressed in 47 out of 59 (79.66%) specimens. No statistical correlation could be found between VEGF overexpression and age, clinical stage, degree of osteolytic lesions, types of monoclonal protein, hemoglobin concentration, platelet count, serum concentration of creatinin, calcium and albumins, the extent of bone marrow infiltration, histological grade and proliferative activity (measured with Ki-67 immunoreactivity). In addition, no signif- icant difference regarding overall survival was found between VEGF positive and VEGF negative cases (29 months vs. 34 months, v=0.5). Median MVD was 15 (range: 1-89). We found significant correlation between MVD and histological grade, the extent of bone marrow infiltration and proliferative activity. Although MVD showed prognostic impact on overall survival in univariate analysis (p=0.009), multivariate analysis identified only age, hemoglobin concentration and proliferative activity as independent prognostic factors. Conclusions. The upregulated VEGF is seen in plasma cells in the majority of myeloma cases. However, the relationship between this finding and pathogenesis of the disease still remains to be established. The microvessel density can predict poor survival in myeloma and be helpful in choosing optimal therapeutic modality in every individual patient.

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**0760**

**CLONOGENIC CAPACITY OF BONE MARROW CELLS IN PATIENTS WITH MULTIPLE MYELOMA: THE INFLUENCE OF ARSENIC TRIOXIDE AND BORTezOMIB ON THE PROLIFERATION OF CFU-F AND CFU-GM**

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Arsenic trioxide (As2O3) and bortezomib were tested as therapeutic agents for a variety of malignancies. The aim of our study was to investigate in vitro effects of As2O3 and bortezomib on clonogenic capacity of haematopoietic and mesenchymal progenitor cells in patients, newly diagnosed multiple myeloma and patients with multiple myeloma resistant to standard chemotherapy. Materials and methods. Bone marrow samples were obtained from 24 patients with multiple myeloma: 10 before treatment and 14 patients resistant to standard chemothera- py, 11 females and 13 males, 16 with IgG, 6 with IgA, 1 with IgD, 1 with B) myeloma. Monoclonal cells (MNC) were cultured without As2O3 or bortezomib and with As2O3 at 0.2, 0.5, 1, 2 and 4 μM or bortezomib at a concentration of 10 and 20 ng/mL. MNC were plated in a standardized methylcellulose medium (MethoCult 4434, StemCell Technologies) and MesoCult (StemCell Technologies). Colony forma- tion of haematopoietic progenitors (CFU-GM and BFU-E) and mesenchymal progenitor colonies was assessed on day 14 of cultures. CFU-GM, BFU-E and CFU-F expressed as the percentage of decrease versus control and the mean and standard deviation (SD) of colony inhibition for each concentration of As2O3 or bortezomib were calculated across all samples. Results. In all patients with resistant myeloma and 2/3 of newly diagnosed patients we
observed an increased number of mesenchymal progenitors in cultures. As6O3 and bortezomib caused reduction of CFU-GM and BFU-E formation after 14 days of incubation to 1% and 0.5% of control values respectively. Formation of CFU-F was completely inhibited by As6O3 and bortezomib. Conclusions. Our data clearly demonstrate that in vitro conditions exposure to As6O3 or bortezomib even in low concentration is able to induce growth inhibition of haematopoietic progenitor cells in patients with multiple myeloma. As6O3 and bortezomib inhibits completely formation of mesenchymal progenitor cells in this group of patients. A combination of direct toxicity against leukemic cells with proapoptotic activity of bortezomib or As6O3 may be the optimal characteristic of a successful antmyeloma agent in particular in patients with increased number of CFU-F before treatment.

0761
CHARACTERIZATION OF THE PLASMA CELLS OF MULTIPLE MYELOMA BY SERIAL ANALYSIS OF GENE EXPRESSION
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Backgrounds. In the last years, several large-scale gene expression studies with array based hybridisation have been performed in multiple myeloma (MM). Many genes expressed with the disease have been identified. However, the molecular mechanisms involved in the disease are still not completely elucidated. More recently, the serial analysis of gene expression (SAGE) method has allowed the global analysis of genes expressed in a determined cell or tissue. However, to the best of our knowledge, no consistent studies in plasma cells of MM have already been performed using the SAGE method. Aim. The aim of this study was to characterize the plasma cells of MM by SAGE. Methods. Purified normal plasma cells (PNPC) differentiated from bone marrow B cells of a healthy individual and purified neoplasic plasma cells from a newly diagnosed MM patient were obtained by magnetic sorting in a column, using the CD-138 antibody Macs microbeads (MACS, Miltenyi Biotec, Germany). Both SAGE libraries, PNPC and MM, were made by SAGE kit (Invitrogen, Life Technologies, USA), in accord with the manufacturer procedures. The expression of a group of genes arbitrarily selected were further investigated by quantitative polymerase chain reaction real-time (qRT-PCR) in the sample of SAGE MM and in other samples of MM patients, in comparison with SAGE PNPC sample, with the purpose of verify the reliability of the results obtained by SAGE. The functional classification of genes was performed according to the Gene Ontology Consortium. Results. We generated, after SAGE, 218,000 and 1,360 unique tags, respectively. In the comparison of both profiles, 476 genes or annotated sequences, and 30% corresponded to tags that may correspond to tags that may be novel transcripts. The expression of 8 up-regulated genes (CCND1, DUSP1, FOS-B, IGHG3, IGKC, V-FOS, V-JUN, PRDM2), 5 down-regulated genes (CD19, CD40, EEF1D, FCER2, IL6-ST rRNAseq1) and two normally expressed genes (B2M e XBP-1) on MM library were evaluated by qRT-PCR in the SAGE MM and in other samples of MM patients. Similar mean expression values were found in both materials. A distinct mean expression value of the PRDM2 gene (109.51 in MM library vs 1.00 in MM samples) was the unique discordant result. The functional classification of genes revealed abnormal expression of genes involved in transcription, signalling, cell proliferation and apoptosis. Conclusions. We identified abnormal expression of genes involved in fundamental plasma cells progression and survival, which may contribute to the comprehension of MM pathophysiology, and to the identification of new targets for MM therapy.

0763
RENAL IMPAIRMENT IN MULTIPLE MYELOMA PATIENTS FOLLOWING Zoledronic acid or Ibandronate Treatment
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Backgrounds. Although bisphosphonates prevent skeletal complications, agents differ with respect to renal safety. Ibandronate (IB) is a single-nitrogen, noncyclic bisphosphonate that has shown a renal safety profile comparable to placebo in phase III trials. This retrospective study aimed to compare renal impairment rates in multiple myeloma (MM) patients treated with IB or zoledronic acid (ZO). Methods. Medical records in a German oncology clinic from May 2001 to December 2005 were retrospectively reviewed. Creatinine measurements were analyzed from baseline (before ZO or IB treatment) to last evaluation for each patient. Renal impairment was defined as (1) a serum creatinine (Scr) increase of ≥0.5 mg/dl or ≥1.0 mg/dl from baseline values of <1.4 mg/dl or <2.6 mg/dl, respectively, or (2) a ≥25% decrease in glomerular filtration rate (GFR, abbreviated MDRD formula) from baseline. Patients treated sequentially with both ZO and IB were included as separate observations. Multivariate analyses were conducted using the Cox proportional hazards model and the Andersen-Gill (A-G) extension of the Cox model for multiple-occurrence. In patients at diagnosis n=67, 69 received ZO and 40 received IB, with 25 patients receiving both drugs. Compared with IB, the ZO group had a significantly better baseline renal function (mean Scr 1.01 vs 1.19 mg/dL; p=0.0002; mean GFR 75.9 vs 57.3, p=0.0002). Data analysis showed that ZO treatment increased the relative risk (RR) of renal impairment by ~3-fold compared with IB (renal impairment rates: ZO 5.7% vs IB 0.6%, RR=3.5, p=0.02; IB 23.4% vs 24.3%, RR=2.6, p=0.0002 [GFR]). The incidence rate of renal impairment was higher for ZO than IB (Scr 1.03 vs 0.18 events per person-year, p=0.0001; GFR 2.93 vs 0.89 events per person-year, p=0.0001). IB
patients who switched from ZO treatment had a significantly higher risk of renal impairment than IB monotherapy patients (renal impairment rates: switchers 40.9% vs monotherapy 6.7%, RR=6.1, p=0.023 [SCR]; 63.6% vs 26.7%, RR=2.4, p=0.029 [GFR]) but experienced a significant trend towards improved renal function during the IB treatment period after a significant trend towards renal deterioration in the ZO treatment period. Multivariate analysis using the Cox proportional hazards model and the A-G model for multiple-event analysis consistently found significantly higher hazards ratios for ZO over IB, after adjusting for differences in characteristics between the two treatment groups. (SCR: Cox=4.2, p=0.016; A-G=0.0, p<0.0001; GFR: Cox=4.2, p=0.001; A-G=3.6, p<0.0001).

Conclusions. In this retrospective review, MM patients were significantly more likely to experience renal impairment with ZO than with IB. Among IB patients, those previously treated with ZO had a higher risk of renal impairment than monotherapy patients. A prospective randomized study is warranted for further validation.

**0764**

**LOW DOSE BORTEZOMIB, DEXAMETHASONE, THALIDOMIDE PLUS LIPOSOMAL DOXORUBICIN IN RELAPSED AND REFRACTORY MYELOMA**

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**Background.** Bortezomib, a proteasome inhibitor, has proven effective in phase II/III clinical trials. Due to antracyclines and bortezomib possessable interactivity, their combination is attractive. Liposomal doxorubicin has a longer half-life than standard doxorubicin, lower cardiac toxicity and comparable efficacy. It can be safely used in elderly patients or patients with organ dysfunction.

**Methods.** pegylate liposomal doxorubicin (Myocet) to low dose bortezomib, dexamethasone and thalidomide regimen (LD-VTD) we previously tested in the therapy of multiple myeloma (MM). Aims: to evaluate the efficacy of Bortezomib in patients with refractory relapse MM treated in our Department from December 05 to January 06. Patients and Methods. We have included 34 patients with relapsed MM that had been treated with one or more lines of therapy, (VBCM/VBAD, VAD, PBSCT one or tandem, TACIDEX, radiotherapy). Bortezomib (1.3 mg/m² twice weekly for 4 weeks, followed by 2 weeks on, 1 week off) was administered for a maximum of 24 cycles. The time to response was from the date of the first administration of bortezomib to the first evidence of response. For a maximum of 4 cycles. The time to response was from the date of the first administration of bortezomib to the first evidence of response. Results. 20 pts, median age 64 yrs, entered the study: 4 stage IIA, 14 IIIA and 2 IIIB, β2microglobulin >4 mg/L in 8 (40%). FS >5 in 7 pts and grade 2 FN in 8. Nine (45%) were primary refractory and 11 R/R. Median time from diagnosis was 6 years. A median of 4 (range 2-6) were the prior therapy lines. All pts had previously received thalidomide plus dexamethasone and 6 (1 refractory, 5 relapsed) the LD-VTD regimen. At February 28th 2006, 38 cycles (median 2/pts) were delivered. Thirteen (65%) pts discontinued due to toxic effects. Haematological toxicity presented at day 14-17 and lasted for a maximum of 4 days: grade 2 neutropenia in 3 pts, grade 4 thrombocytopenia in 5. One patient thrombocytopenic at study entry, had a grade 4 gastric haemorrhage. Two pts had pneumonia, 2 HZV infection. Other adverse events of grade >1 were fatigue (50%), nausea (60%), diarrohea (15%), alopecia (10%). None experienced progression of FN. No case of DVT or cardiac failure was recorded. Except for 3 pt, all were treated on an outpatient basis. Sixteen pts are valuable for response: 2 were removed from the study for progression of disease, one had stable disease. Thirteen responders: 1 CR, 8 SCR, 5 PR, 1 MR (ORR 81%). Median time to best response 1,2 months (range 1-3). After a median follow-up of 7 months, 19 patients were alive. Conclusions. toxicity was acceptable and most pts were treated on an outpatient basis. Cytopenias recovered within the rest period. A significant neurological toxicity was not recorded, probably due to the lower dose of bortezomib applied. Although an adequate follow-up is needed to draw any conclusion about TTP and OS, this regimen appears very effective as we achieved an ORR of 91% vs 55% previously attained (Ciolli et al, 2006).

**ANALYSIS OF RESPONSE AND FOLLOW-UP IN RELAPSED REFRACTORY MYELOMA RECEIVING BORTEZOMIB**

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**Background.** Bortezomib, a boronic acid dipeptide a novel targeted therapy is a proteasome inhibitor that has been shown to be effective in the therapy of multiple myeloma (MM). Aims: to evaluate the efficacy of Bortezomib in patients with refractory relapse MM treated in our Department from December 05 to January 06. Patients and Methods. We have included 34 patients with relapsed MM that had been treated with one or more lines of therapy, (VBCM/VBAD, VAD, PBSCT one or tandem, TACIDEX, radiotherapy). Bortezomib (1.3 mg/m² twice weekly for 2 weeks on, 1 week off) was administered for a maximum of 21 days in an outpatient regime were administrated. The response was evaluated according to the criteria of the Consensus Report Scientific Advisor International Myeloma Foundation 2003. CR: without symptoms, no monoclonal component (MC) detected by immunofixation electrophoresis (IFE) (Sebia standardized procedure), PR: reduction of MC >50%, Minimal Response (MR): reduction of MC 25-50%, Clinical response (Clin R): no clinical symptoms, and non-response (NR). Adverse effects were registered. Results. 34 patients (males 47%, mean age 65 years, 65), over 65 years (50%). Type IgG: 11 (6 II-A, 5 III-A); IgA: 12 (7 II-A, 5 III-A); B: 7 (3 II-A, 4 III-A), B1gG: 2 II-A, 1 III-A, B1gA: 1 IIIA. Previous therapy: 1 schedule (5 15.4%), 2 (30.7%), 3 (1750%), 4 (138%). For the analysis 36 patients were valuable. Response were reached in 76.9%, (CRPR 65.4%), (CR46.1%), (CR 19.4%). Regarding CR (Clin R) 86.1%, Mean courses to reached response 3.6. No relation to response or presence or not chromosomal aberrations were observed. At 24 months on follow-up 7 patients had dead (20.6%) and 11 (42.3%) maintained response without therapy. In 11 patients (42.3%) a combination of Bortezomib+Dexa or Melphalan were administrated by relapse or progression. Adverse events. Thrombocytopenia (grade 3: 5, grade 1: 1) 46.1%, fatigue 5 (38.5%), peripheral neuropathy 4 (30.8%), constipation 3 (23%), diarrhea 2 (15.4%), ZHV 2 (15.4%), pneumonia 2 (15.4%), pyrexia 1 (7.6%), hyponatremia 2 (15.4%), grade 3 leukopenia 1 (7.8%). In 2 patients (15.4%) the therapy was disrupted by toxicity. Conclusions. Bortezomib in monotherapy induce a high rate of response (76.9%) in refractory MM. The response is achieved in the first 4 courses. It is recommendable to make combinations after the 4th course of Bortezomib if response does not achieved. No severe adverse effects have been observed with an incidence of reversible haematological side effects in 46.1% and mild non-haematological side effects in 52%.

**TUMOR-HOST INTERACTIONS IN THE BONE MARROW OF MULTIPLE MYELOMA PATIENTS: ANALYSIS OF CLINICAL RELEVANCE AND PROGNOSTIC SIGNIFICANCE**

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Institute of Hematology, Belgrade, Serbia and Montenegro

Complex pathogenesis of multiple myeloma (MM) includes a functional interplay between the myeloma cell, cells and microenvironment resulting with the interaction of various cytokines, their receptors and adhesion molecules. The aim of study was to analyze prognostic significance of different tumor-host interactions in the bone marrow (BM) of MM patients (pts) by immunohistochemical markers of angiogenesis, osteoclastogenesis and sensitivity to the IL-6. The study included 80 newly diagnosed MM pts (55 male and 25 female, age 50-60 years, range 35-75). According to the clinical stage (CS, Salmon&Durie), distribution of MM pts was as follows: 18 pts, II22pts, III30pts. Renal impairment existed in 17pts. There were 5pts with IgG monoclonal (M) protein, 12pts with IgA and 12pts with secretion of kappa/lambda light chain. None secretary MM was diagnosed in pts. Regarding IPI score, the group included 19pts in stage1, 13pts in stage2, and 29pts with IPI 3. All patients were treated with conventional chemotherapy. In order to analyze microvesel density (MVD), BM vessels were visualized by immunohistochemical staining for CD34. The number of vessels per 400x high power field (HPF) was counted in the area of the most dense vascularization. All samples were stained with an antihistochemical expression of FGFR-3, OPG, RANKL, and gp130. The intensity of these stainings was graded as weak (0-30% positiveness), moderate (31-60% positiveness), and strong (>60% positiveness). Control specimens were obtained from pts without hematological malignancy.
A multicenter retrospective analysis of adverse events in Korean patients using bortezomib for multiple myeloma

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Backgrounds. The proteasome inhibitor bortezomib has demonstrated clinical activity in patients with multiple myeloma (MM). Adverse events including thrombocytopenia and peripheral neuropathy affected 30% to 60% of patients overall, and interrupted therapy in 10% to 20%. There is no prior toxicity data available for Asian patients using bortezomib for MM. Aims. To evaluate the pattern of adverse events in patients treated with bortezomib for their MM. Methods. We reviewed the clinical records of patients with the diagnosis of MM from 25 centers in Korea using the NCI Common Toxicity Criteria version 3.0. The included patients were treated with bortezomib alone or in combination with other agents including thalidomide. Results. Nineteen patients with MM were treated; patients had a median age of 60 years (range: 42-77). The median number of previous treatments was 3 (range: 0-10), 39% of patients had been treated with four or more major classes of agents including thalidomide (67%) and autologous stem cell transplantation (51%). Regimens included bortezomib only in 35 (40%), bortezomib plus dexamethasone in 34 (39%), and bortezomib plus a thalidomide-containing regimen in 23 (24%) patients. The analysis of patient response to therapy revealed: CR + nCR in 31 (38%) and PR in 30 (32%), for an ORR of 65% in 93 patients. The most common adverse events reported were thrombocytopenia (47%), sensory neuropathy (42%), anemia and leukopenia (both 31%). Thirteen patients (14%) stopped therapy due to adverse events; neuropathy in 8, infection in 4 and diarrhea in 1 patient. A neuropathy, more than grade 2, was more frequent in patients who received 4 or more prior therapy regimens (17/57) compared to those receiving 3 or less (1/30). Also combination of thalidomide and bortezomib increased the risk of neuropathy (56%). Infection was the most common cause of death (14/93) and in 3 patients the cause was not clear. Conclusions. The incidence of thrombocytopenia and neuropathy were similar, however gastrointestinal toxicities were relatively low in Korean patients compared to western studies. Significant neuropathy was associated with the number of prior regimens and combination with thalidomide. These findings provide useful information for clinicians and patients using bortezomib.

Frequency and distribution of trisomy 11 in multiple myeloma patients: relation with overexpression of CCND1 gene and t(11;14) translocation

T. Guglielmelli, T. Guglielmelli, E. Giugliano, S. Cappia, M. Papotti, G. Saglia

Univ. of Turin and St Luigi Hospital, Orbassano, Italy

Backgrounds. CCND1 is an established oncogene located on chromosome band 11q13 for which genomic rearrangement or amplification leading to overexpression of the cyclin D1 protein is commonly found in a high proportion of patients suffering from multiple myeloma. The cyclin D1 protein is an important regulator of the cell cycle and its expression is linked to diagnosis and survival of patients with myeloma. In mantle cell lymphomas, where the cyclin D1 protein is present in almost all cases and is activated by the characteristic t(11;14) translocation. Also 50% of human breast cancers exhibit cyclin D1 protein overexpression: in 20% of these tumours amplification of the 11q13 region is present but in the remaining cases overexpression cannot be explained by copy number increases. The study by the authors investigating whether the overexpression of this gene occurs in patient with MM and which may act as potential therapeutic target for clinical intervention.

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head and neck) and melanomas, cyclin D1 is activated by gene amplification and is associated with poor prognosis. CCND1 overexpression have also been found in 25-50% of multiple myeloma (MM) cases. A molecular classification of MM, namely TC classification, stratifies patients into five groups (TC1-TC5) based on the presence of the recurrent IgH chromosomal translocations and cyclins D expression. Patients overexpressing CCND1 can be divided into two groups: TC1, characterized by the t(11;14) or t(6;14) translocation with overexpression of CCND1 or CCND3 and a non hyperdiploid status and TC2, with low to moderate levels of CCND1, absence of any primary IGH translocation and a hyperdiploid status. Aims. To assess CCND1 gene and cyclin D1 protein overexpression in a series of primary MM patients, to explore its relationship to the presence of the t(11;14), and to evaluate frequency and distribution of trisomy 11 in the different TC groups. Methods. fluorescence in situ hybridization (FISH) analysis with specific probes for CCND1 gene amplification (probe mixture of cyclin D1 band 11q13 - CEP 11 bands 11p11-q11) and t(11;14)(q13;q32) were performed on CD138-purified plasmacytomas from bone marrows of thirty MM patients at diagnosis. Cyclin D1 protein expression and intensity was evaluate by immunohistochemistry. Results. FISH analysis revealed CCND1 overexpression in 14/30 cases (46.6%) and the presence of the t(11;14) translocation in 9/30 cases (30%) (Table 1). Patients with evidence of the t(11;14) showed strong nuclear staining for cyclin D1 (TC1 group) and 8 out 9 demonstrated CCND1 overexpression. The remaining 6 out 18 cases with increased CCND1 gene copy numbers lacked the t(11;14) and showed low to negative levels of cyclin D1 protein (TC2 group). Globally, the frequency of trisomy 11 was 40% (12/30 patients). It was demonstrated in 3 out 9 cases carrying the t(11;14) (TC1), 5 out 6 overexpressing CCND1 without the translocation (TC2) and 4 out 15 negative for both alterations (TC3-TC5). Conclusion. In our data, trisomy 11 don’t seems to cause directly overexpression of CCND1 as it is present in 4/15 patients without overexpression of CCND1 and in 3/9 patients carrying the t(11;14). One patient belonging to the TC2 group, overexpresses CCND1 and lacks both trisomy and translocation suggesting that cyclin D1 can be dysregulated by additional mechanisms. In TC2 group trisomy 111 probably may be considered as a recurrent polynomy of the hyperdiploid status.

### Table 1.

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*In the presence of CCND1 overexpression, the number of copies for each gene is indicated. NP: not performed; MC: monoclonal component. §IHC score ++++ >75% tumor cell positive; +++ 50-75% tumor cells positive, 25-50% tumor cells positive, +10-25% tumor cells positive.
tation none of the patients had peripheral neuropathy > grade 1. Patients were scheduled to receive bortezomib 1.3 mg/m² IV (days 1, 4, 8 and 11) every three weeks for eight cycles and dexamethasone was planned to be added at a dose of 20 mg every other day (days 2, 5, 9, 12), if no signs of response were observed after the 1st cycle. Actually, 27 patients received dexamethasone and the 2 patients with plasma cell leukemia received thalidomide in addition. Results. Two patients in terminal resistant disease died prematurely and 3 patients are still receiving the 2nd cycle (thus, 5 patients are not evaluable at present). Overall, 39 out of 47 evaluable patients responded (83%) (3 complete remission [CR], 5 near CR, 25 partial response, 6 minimal response). The median time to response was 42 days. Within a median follow-up of 8 months (2-160), 16 (34%) patients relapsed and 8 patients (15%) died, 7 of disease and 1 of unrelated cause. In the majority of patients, non-neurologic toxicity was mild and reversible including fever, fatigue, gastrointestinal symptoms and hematologic toxicity. The most severe side effect was peripheral neuropathy which developed in 60% of patients. Neuropathy included ataxia, caustic pain and sensory disturbances and resolved after a median time of two months after discontinuation of Bortezomib. In most patients, treatment was reduced or stopped because of peripheral neuropathy but all responding patients completed at least 4 cycles. Conclusion. Bortezomib therapy alone or in combination with low dose dexamethasone produces rapid responses in relapsed and refractory MM. Early relapses are frequent. Neuropathy is the most important adverse reaction and lead to dose reduction or discontinuation of treatment.

**0772**

**BORTEZOMIB AS A SINGLE AGENT IN REFRACTORY/RELAPSED MULTIPLE MYELOMA RESULTS OF CZECH MYELOMA GROUP (CMG)**

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Summary. The efficacy of single agent bortezomib in the treatment of refractory multiple myeloma has been shown repeatedly. We summarize the results of bortezomib therapy in Czech Republic. 32 patients with a median age 61 years (33-84) with refractory/relapsed myeloma were scheduled to receive Velcade 1.3 mg/m², on days 1, 4, 8 and 11 every three weeks for eight cycles and dexamethasone was planned to be added at a dose of 20 mg every other day (days 2, 5, 9, 12), if no signs of response were observed after the 1st cycle. Actually, 27 patients received dexamethasone and the 2 patients with plasma cell leukemia received thalidomide in addition. Results. Two patients in terminal resistant disease died prematurely and 3 patients are still receiving the 2nd cycle (thus, 5 patients are not evaluable at present). Overall, 39 out of 47 evaluable patients responded (83%) (3 complete remission [CR], 5 near CR, 25 partial response, 6 minimal response). The median time to response was 42 days. Within a median follow-up of 8 months (2-160), 16 (34%) patients relapsed and 8 patients (15%) died, 7 of disease and 1 of unrelated cause. In the majority of patients, non-neurologic toxicity was mild and reversible including fever, fatigue, gastrointestinal symptoms and hematologic toxicity. The most severe side effect was peripheral neuropathy which developed in 60% of patients. Neuropathy included ataxia, caustic pain and sensory disturbances and resolved after a median time of two months after discontinuation of Bortezomib. In most patients, treatment was reduced or stopped because of peripheral neuropathy but all responding patients completed at least 4 cycles. Conclusion. Bortezomib therapy alone or in combination with low dose dexamethasone produces rapid responses in relapsed and refractory MM. Early relapses are frequent. Neuropathy is the most important adverse reaction and lead to dose reduction or discontinuation of treatment.
0774
Dexamethasone induces apoptosis ex vivo in chronic lymphocytic leukemia cells with either unmutated IgVH genes or high ZAP-70 expression
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Background. Patients with chronic lymphocytic leukemia (CLL) and unmutated IgVH genes or high ZAP-70 expression have poorer prognosis than those with mutated IgVH genes or low ZAP-70 levels. This is in part related to the resistance of unmutated and ZAP-70 positive cases to sis than those with mutated IgVH genes or low ZAP-70 levels. This is in part related to the resistance of unmutated and ZAP-70 positive cases to treatment agents that induce apoptosis in a p53-dependent manner. It has been suggested that corticosteroids are active in CLL through p53-independent pathways. Aims. To analyze the ex vivo response to dexamethasone in CLL cells according to the IgVH mutational status and ZAP-70 levels, and the expression of different glucocorticoid receptors in CLL cells. Methods. Frozen lymphocytes from 60 patients with CLL were analyzed for ZAP-70 expression and IgVH mutational status (n=44). Cells were cultured and treated using Fludarabine (5 µg/mL), Mitoxantrone (0.5 µg/mL), FCM (fludara 1 µg/µL, maphosphamide 1 µg/mL and mito 0.5 µg/mL), FM (fludara 1 µg/mL and mito 0.5 µg/mL), Dexamethasone (5.2 µg/mL) and FMD (fludara 1 µg/mL, mito 0.5 µg/mL and dexam 5.2 µg/mL). Cell viability and apoptosis were determined by annexinV binding and FACSscan analysis at three different time points and conditions (0 and 24h without treatment, and 24h with each treatment). The expression of glucocorticoid receptor (GR) isoforms α, β and γ was analyzed by Quantitative RT-PCR in 20 cases. Results. Dexamethasone-induced apoptosis was significantly higher in samples with unmutated IgVH genes and/or high ZAP-70 expression (≥20%) than in those with mutated IgVH genes and/or low ZAP-70 expression (median cell viability 65% vs. 81%, respectively, p<0.001). In contrast, the highest cell mortality induced by mitoxantrone was observed in samples with IgVH mutations or low ZAP-70 expression (p=0.009). Median cell viability was 56.1% for FM vs. 34.3% for FMD (p<0.0001) regardless of the IgVH mutational status. No differences in cell viability were found according to ZAP-70 expression or IgVH mutational status after ex vivo treatment with Fludarabine or FCM (p=0.649 and p=0.055, respectively). No relationship was found among IgVH mutational status and the expression of the different GR isoforms. Expression of the three different GR isoforms was also similar in corticosteroids responders and non-responders. Conclusions. In this study, CLL cells with unmutated IgVH genes or high ZAP-70 expression showed a higher cell mortality after ex vivo exposure to dexamethasone than those with mutated IgVH genes or low ZAP-70 expression, with no relationship with the expression of the different GR isoforms. These data give conceptual support to trials aimed at determining the role of dexamethasone in the treatment of patients with CLL and poor prognostic features or resistant to Fludara.
0777
ZAP-70 EXPRESSION IN NEOPLASTIC CELLS AND T LYMPHOCYTES OF B- CLL PATIENTS: A REPRODUCIBLE METHOD FOR DETECTION USING FLOW CYTOMETRY
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Background. Prognostic stratification in B-CLL is critical to design informative therapeutic trials. The best combination of biological and clinical parameters for patient classification in prognostic groups should include Zap-70 expression, which correlates with lack of somatic mutations of immunoglobulin V-H genes. Determination of Zap-70 expression by flow cytometry is attractive due to wide accessibility and speed, but is hampered by lack of standardization. Aim. The aim of this study was to identify the best strategy for flow cytometric analysis of Zap-70 expression in B-CLL cells, and to compare it with other prognostic factors. Methods. Zap-70 expression was determined in 136 samples from 59 patients (19 males, 20 females; median age 65.3, 31-85 years), including 23 stage A and 16 stage B or C. Zap-70 expression was determined within 24 h of sample drawing. In 15 patients the determination was performed before treatment. Anti-CD19, CD20, CD22, CD23, FMC7, CD79b, CD38, CD8, CD5, Zap-70 (PE-labeled), K and L fluorescent monoclonal antibodies were used, and a minimum of 5000 events were acquired for each sample. We defined B-CLL cells expressing Zap-70 with intensity equal or higher than T cells. If Zap-70 was expressed in more than 20%, tumor cells were considered positive. To reduce inter-observer and technical variability, we further calculated the ratio between the median fluorescent intensity (MFI) for Zap-70 in B-CLL and T cells. The relationship with CD38 expression (positive if present in more than 50% of neoplastic cells) and genetic abnormalities (deletions of 13q, 11q, 17p and/or trisomy 12, evaluated by FISH) was determined using the χ² test. Time-to-first-treatment was calculated using the Kaplan-Meier method and the influence of Zap-70 expression evaluated by the log-rank test. Results. With a median follow-up of 54.7 (0-145) months, only one patient died. Median time-to-first-treatment was 11.3 (0-132.9) months, with 7 patients receiving fludarabine-based regimens, 12 alkylating agents, and 2 anticyclines (global response rate 62%). Patients were divided into 3 groups according to the presence of the lowing cytogenetic findings: del17p and/or del11q (20.5%), trisomy 12 or no abnormalities (38.5%), and del13q (23.1%). Seventy-four percent were Zap-70 positive, while only 36% were CD38 positive. Zap-70 positivity was unrelated to Binet stage, CD38 expression and cytogenetic findings (p=0.335). Time-to-first-treatment was similar in Zap-70 positive and negative patients (p=0.99). However, when the ratio between MFI of Zap-70 in B-CLL and T cells was used, we found that patients with Zap-70 higher than 0.4 had a prolonged time-to-first-treatment as compared to patients with lower ratios (p=0.03). Conclusions. Determination of Zap-70 expression using the ratio between Zap-70 MFI in tumor and T cells is a reproducible method for Zap-70 evaluation in B-CLL, by simultaneously providing an internal control for the fluorescence intensity of positive cells and reducing the inter-observer and technical variability.

0778
A PHASE II STUDY OF THE COMBINATION OF ALEMTUZUMAB AND PENTOSTATIN IN PATIENTS WITH T-LYMPHOCYTIC MALIGNANCIES
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Background. Mature T-cell lymphoid neoplasms are clonal disorders of post-thymic T-cells that are considered separately from precursor T-cell leukemias. These neoplasms are generally refractory to traditional chemotherapy regimens and as such, the prognosis for most patients is poor; novel therapeutic strategies are needed. Alemtuzumab and pentostatin have reported response rates of 50% to 75% when used individually, but most responses are partial or of limited duration. Immunosuppression and risk of opportunistic infections are their principal overlapping toxicities. Aims. To determine the feasibility and efficacy of the combination of alemtuzumab and pentostatin for the treatment of T-lymphoid malignancies. Methods. We have treated 13 patients with T-lymphoid malignancies (7 T-PLL, 1 ATL, 1 T-ALL, 2 γ/γ T-cell leukemias, 2 T-GLL) with the combination of alemtuzumab (ab 30 mg IV three weekly for up to 3 months) and pentostatin 4 mg/m² weekly for 4 weeks followed by alternate weekly administration for up to 6 months. Prophylactic antibiotics including valacyclovir, trimetho-

prim/sulfamethoxazole, and fluconazole (or equivalents) were administered during the treatment and for 2 months after completion. Results. The median age of patients was 57 years (range 22-80 years), median white blood count was 60.5×10⁹/L (range 0.6-279.5×10⁹/L), median serum β-2M was 4.1 mg/L (range, 2.2 to 10.8 mg/L). Four patients had splenomegaly (1-6 cm), and 5 lymphadenopathy. Eight had prior therapy (median 5, range 1 to 6 regimens). Eight patients have responded (7 CR, 1 PR) for an overall response rate of 62%. Monoclonal T-cell receptor chain gene rearrangements were detected by PCR in 7 patients and became negative in 2 of 4 evaluable patients in CR. Median response duration is 5+ months (range 0.25+ to 13+ months).

0779
WTNSB EXPRESSION: A NEW POTENTIAL PROGNOSTIC MARKER IN CHRONIC LYMPHOCYTIC LEUKEMIA
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Background. ZAP70 and CD38 expression, along with heavy chain (IgH) mutational status, are currently under investigation as predictive markers in chronic lymphocytic leukemia (CLL). Previous data (Desheng et al PNAS 2004) suggested that Wnt signaling pathway contributes to a deficient apoptosis in CLL. Wnt5b is a ligand for members of the frizzled family of seven transmembrane receptors. It may be a signaling molecule which affects the development of some regions of tissues. Correlation of ZAP70, CD38 expression and IgH status with Wnt5b expression. Method. This retrospective study was conducted on 14 newly diagnosed and 9 relapsed CLL patients and on 18 healthy donors. We used the ABI PRISM 7000 Real Time platform to analyze Wnt5b expression, which was normalized against healthy donors median expression (P/H). We also evaluated CD38 (Ibrahim et al Blood 2001), ZAP70 expression (Crespo et al N Engl J Med 2003) and IgH rearrangement (Theriault et al Mod Pathol 2000) as previously described. Results. All healthy donors and 15 CLL patients showed Wnt5b expression, while 8 CLL patients were negative. After a median follow-up of 21.5 months (range 6-29), 6 patients were in complete remission, 11 were in stable disease (SD) and 6 had progressive disease (PD). 14/15 patients (95%) with Wnt5b expression at diagnosis were in PD or SD (p=0.01 vs patients in CR) at the end of follow-up. All patients who achieved a CR after therapy had no or very low Wnt5b expression at diagnosis (medi-
an P/H=0, range 0.0-0.26), while SD or PD patients had a median P/H ratio higher than 0.3 (range 0.10-1.2, p=0.01). ZAP70 was evaluated on 20/23 patients. 15 of them were ZAP70+ (75%) and 5 were ZAP70- (25%). 9 patients were ZAP70+/Wnt5b+; 88% of them had a SD or PD; 3 patients ZAP70-/Wnt5b+ showed no CR. Among the 6 patients ZAP70+/Wnt5b- (80%) obtained a CR. Both patients showing ZAP70-/Wnt5b- achieved a CR. 21% of patients were also evaluated for CD38 expression; 62% (18/21) were CD38- and 70% of them were Wnt5b+. 38% were CD38+ and 50% of them were Wnt5b+. IgH status was determined in 12 patients before and after treatment; 9 of them (75%) maintained a monoclonality and 3 (25%) became polyclonal. 67% of monoclonal patients at revaluation were Wnt5b- at diagnosis, 64% of them achieved PD or SD, while 38% of polyclonal patients were originally Wnt5b+. Conclusion: In our CLL population, Wnt5b diagnosis expression suggests an association with poor outcome. Among ZAP70- patients (good prognosis) Wnt5b expression seems to further select patients with a worse prognosis. We are prospectively evaluating a larger patient population to confirm the predictive potential role of this novel biomarker in a multivariate analysis. The availability of these novel biologic prognostic indicators might be of relevance for future risk-adapted treatments.

0780 T CELL SUBSETS AND CMV INFECTION IN CLL: ASSOCIATIONS WITH IGVH MUTATIONAL STATUS, GENE USAGE AND CHROMOSOMAL ABERRATIONS

H.D. Alexander, C. Matthews, M.A. Catherwood, T.C.M. Morris

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Background. Chronic lymphocytic leukaemia (CLL) is characterised by a profound dysregulation of the host’s immune system. This results in recurrent infections and an increased incidence of autoimmune diseases, suggesting T-cell dysfunction. Furthermore, the frequent use of potenti- tally more immunosuppressive therapies such as fludarabine and alemtuzumab, has also increased the incidence of recurrent infections, such as cytomegalovirus (CMV), in CLL. CMV is a member of the herpes family of DNA viruses and arguably causes the most morbidity and mortality of any herpes virus. CMV establishes life-long latent infection without clinical disease in immunocompetent individuals, but can cause severe illness in newborns, transplant recipients, cancer and HIV patients. Aims. This study was designed to determine the frequency of CMV DNAemia in CLL patients, in comparison to normal age and gender matched controls. Associations were sought between evidence of CMV DNAemia and elevated T-lymphocyte subset numbers to determine if T-subset expansions could be used as surrogate markers of CMV infection. IgVH mutational status, VH gene usage and chromosomal aberrations were determined to ascertain if CMV DNAemia occurred more fre- quently in patients with markers of poor outcome. Methods CMV DNA copy number was measured using KQ-PCR CMV kit on a light cycler. IgVH mutational status and VH gene usage were determined using BIO- MED-2 primers and protocol, while the presence of adverse cytogeneti- cals was ascertained using interphase FISH technique. Results CMV DNAemia was detected in 95/113 (84%) of CLL patients. Six of these had DNA copy number >1 ×10⁵, while 18/113 (16%) had no detectable viral DNA. CMV infection was significantly more common in CLL than normal age and gender matched controls. Additionally, the presence of elevated CMV DNA copy number was not dependent on prior exposure to chemotherapy agents. No significant associations between CMV DNAemia and T cell subsets or markers of poor prognosis were noted. However, signif- icant associations between high absolute cytotoxic T cell (CTLs) counts and poor prognosis chromosomal aberrations, and advanced clinical stage (Binet B/C), were recorded (p=0.017, p=0.026, respectively). Intriguingly, patients with VH8-21 rearrangements were found to have high CMV titres (p=0.049), whilst VH3-7 gene usage was linked to lower absolute T cytotoxic counts (p=0.009). Conclusions. This study demonstrat- ed that T cell subsets could not act as surrogate markers for CMV DNAemia. Significant CD8+ T cell expansions were identified in the cohort of patients with poor prognosis cytogenetic abnormalities and advanced clinical stage. T cell dysfunction in CLL has been suggested to contribute to the aetiology and progression of the disease by being unable to start, maintain and complete an immune response to the malignant B cell and other antigens. Additionally T cells have been shown to produce cytokines that prevent CLL cell entry into apoptosis. This may lead to the accumulation of cytogenetic aberrations and therefore may be involved indirectly in sustaining the tumour. Finally, the intrigu- ing finding of high CMV DNA copy numbers in patients with VH8-21 rearrangements suggests that CMV infection may play a role in the poor outcomes in these patients.

0781 SERUM THYMIDINE KINASE LEVELS CAN IDENTIFY EARLY STAGE B-CLL PATIENTS WITH MUTATED IGVH GENES MOST LIKELY TO PROGRESS

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Backgrounds. Thymidine kinase (TK) is a cellular enzyme which func- tions as part of the pyrimidine salvage pathway involved in DNA syn- thesis. Serum TK levels have been shown to be correlated with survival in many malignancies, including CLL. Aims. This study was designed to investigate associations between TK levels and other prognostic mark- ers, in newly diagnosed Binet stage A patients. Furthermore, the use of serum TK measurement to identify subcategories of disease within those defined by IgVH mutational status, gene usage, CD38 expression, Zap- 70 positivity and chromosomal aberrations, was studied. Methods. Ninety- one CLL patients were recruited to this study. Serum TK levels were measured using a radioenzyme assay. IgVH mutational status and VH gene usage were determined using BIOMED-2 primers and protocol. Recurring chromosomal abnormalities were detected by interphase flu- orescent in-situ hybridisation (FISH). CD38 and Zap-70 expression were determined by flow cytometry and RT-PCR respectively. Results. The 91 patients (53 male, 38 female) had an age range of 37-94 years, median 71 years. Forty-five of the 91 patients were newly diagnosed Binet stage A patients, 31 were previously diagnosed early stage patients and 15 cases had advanced CLL (Binet C/Rai III/IV). Significantly higher serum TK levels were found in IgVH unmutated, compared to IgVH mutated, patients (p<0.001). Elevated TK levels were also associated with CD38 expression (p=0.015), and poor and intermediate progression chromosomal aberrations (p<0.001). A TK level of greater than 8.5 U/L, best identified patients with progressive disease. Within the newly diagnosed group, nine IgVH mutated cases had a TK level of 8.5 U/L, or greater. Closer scrutiny revealed that these patients had either VH3-21 or VH-69 gene usage and/or had short LDT of <12 mths, which are associated with poorer outcomes. Additionally, within the unmutat- ed subgroup of newly diagnosed patients, only one had a TK level lower than 8.5 U/L. This particular patient had not undergone lymphocyte doubling, greater than four years after diagnosis, which was longer than that seen in the remaining unmutated cases, with the highest TK values recorded in patients with a lymphocyte doubling time. Conclu- sions. Unlike IgVH mutational status, TK does not lose predictive power as disease progresses, with the highest TK levels reported in advanced clinical stage. This study demonstrated that determining serum TK lev- el at diagnosis in early stage patients can identify those most likely to progress and therefore require earlier treatment. Furthermore, the vari- ations in disease progression within prognostic subcategories can be pre- dicted by measuring serum TK levels at diagnosis, allowing further refinement of risk stratification. We confirm the efficacy of TK measure- ment in CLL to determine proliferation activity and predict clinical course of this heterogeneous disease.

0782 DIAGNOSTIC POTENTIAL OF CD38 COMBINED WITH ZAP-70 EXPRESSION IN PREDICTING MUTATIONAL STATUS OF IMMUNoglobulin HEAVY-CHAIN VARIABLE REGION IN 450 CHRONIC LYMPHOCYTIC LEUKEMIA CASES

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Backgrounds. Recent advances in the diagnosis and molecular charac- terization of CLL permit improved prediction of disease prognosis, which could result in better management. The best-studied parameters are somatic hypermutation of the immunoglobulin heavy-chain vari- able region (VH), expression of the cellular proteins CD38 and -associ- ation with Zap-70 (ZAP-70). In this study, we investigated the muta- tional status of the VH genes in CLL cells from a series of 450 cases and correlated the results with CD38 expression detected by flow-cytome- try and ZAP-70 using Western blotting. Methods. Determination of Zap-70 was carried out by Western blot. For the purpose of this study, sam- ples showing a negative and weak Zap-70 patterns were collectively assessed. The B-CLL Ig VH gene usage and mutation was determined on cDNA according previously reported methods. The best cut-off point for CD38 or Zap-70 expression for discriminating between mutated and unmutated IgVH status was sought by constructing receiver operating
characteristic (ROC) curves, which were generated by calculating the sensitivity and specificity of these two methods, showed a determined cut-off point. Moreover, the usefulness of CD38 and ZAP expression in identifying VH mutational status was analyzed according to the following standard diagnostic tests: sensitivity and specificity, positive and negative predicted values and accuracy, as well as by Kappa statistic. Results. As a first step, we determined, by ROC curve analysis, 90% as the best cut-off value of both which discriminates between mutated and unmutated cases in CLLs (area under the curve 0.758, p<0.0001). On the basis of standard diagnostic tests, CD38 expression, categorized by 30% cut-off value, had relatively low sensitivity (70%), specificity (77%), positive predictive value (76%) and positive predictive (71%) values in anticipating VH mutational status. Moreover, Kappa statistic revealed that the agreement between CD38 expression and VH mutational status was low although significant (K=0.47, p<0.001). On the other hand, ZAP-70 showed very low sensitivity (57%), high specificity (89%), low positive predictive value (57%), relatively low negative predictive value (72%) and a low, although significant, K statistic (0.47, p<0.001). Furthermore, we combined the value of both tests to evaluate whether both variables provided more precise information in estimating VH mutational status compared to that obtained from each single test. In this regard, we obtained the following results: sensitivity, 90%; specificity, 96%; positive predictive value, 90%; negative predictive value, 76%; k statistic 0.45, p<0.001. Moreover, ROC analysis was also performed to detect the optimal cut-off of Ig V gene rearrangement capable of providing the best sensitivity and specificity with positivity of both CD38 and ZAP-70. The best cut-off value was 1.9% (AUC 0.814, p<0.0001), which is close to the threshold (2%) used to distinguish mutated from unmutated B-CLL. Conclusion/Summary. Our data demonstrated that neither CD38 nor ZAP-70 by themselves had an important impact in anticipating VH mutational status. When CD38 and ZAP-70 were combined, the diagnostic accuracy was improved, meaning that the combined use of CD38 and ZAP-70 could surmount the prognostic value of VH mutational status. This information should be validated on clinical ground.

0784 IDENTIFICATION OF NEW GENOMIC ALTERATIONS IN CLL USING A 32K BAC CGH ARRAY

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Backgrounds. Cytogenetics in CLL is hampered by low mitotic index. Genetic screening for prognostic factors has been routinely performed by FISH for established abnormalities. All of them are unbalanced, and some (e.g., del13q14) are believed to be early events of pathogenesis, whereas others (del17p) may be associated with progression of disease. To get a more detailed profile of genomic alterations in CLL, we applied a genome wide arrayCGH, Aims. To screen the genome of CLL cells for potential new genomic alterations, with a view to understanding novel genomic alterations, with a view to diagnostic and pathogenetic ramifications. To correlate the genomic data with IgVH mutation status and outcome for patients in the Scandinavian phase III study with primary/initial treatment for symptomatic CLL. Methods. Genomic DNA from formalin fixed paraffin embedded tumor tissues was microdissected, amplified and labeled with biotin using a high resolution 32 K BAC array. The array data was analyzed with BioArray Software Environment (http://base.thep.lu.se/). Results. Preliminary analysis of the DNA copy number profiles allowed detection of previously described genomic changes along with identification of novel alterations. The most common alteration among the CLL samples was del13q14 (40%) followed by del11q22 (80%). In 13 out of 28 samples displaying the del13q14 homozygous deletion was implied. The minimal deleted region (MDR) could be mapped to a region of 0.1 Mb encoding the genes DLEU1, DLEU2 and DLEU7. Losses of chromosome 11q spanned from 11q14.1 to 11q23.2 with the peak at 11q22.3. Trisomy 12 was detected in 25% of the samples, in several samples indicating only partial gains. Loss of the 17p arm was also detected, in some cases with a concurrent gain of the 17q arm. Genomic changes were detected in 80% of the CLL samples. For example, losses on 2q56, 5q13.2, 12, 18q21.2 were detected in individual samples. Recurrent gains were mapped to 6p21.3 and 8q21.2. Summary/Conclusions. The high resolution CGH array combines full genome screening with high specificity and allows detection of small lesions. Genomic abnormalities were identified in most patients, including novel and unknown changes, which may be delineated accurately. Validation of novel changes and correlation studies will follow, and may improve our understanding of genomic lesions and their clinical implications in CLL.

0785 EVALUATION OF TRANSFERRIN RECEPTOR 1 AND 2 EXPRESSION AT THE RNA AND PROTEIN LEVEL IN NORMAL B CELLS VS. CHRONIC LYMPHOCYTIC LEUKEMIA

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Transferrin receptor 1 (TR1, CD71) is one of the classical activation markers up-regulated upon B-cell activation. TR2 has significant surface expression on TR1 and also binds transferrin, albeit with lower affinity than TR1. The TR2 gene has two alternatively spliced transcripts (α and β): in normal tissues, TR2-α mRNA is more abundant than TR2-β mRNA. Chronic lymphocytic leukemia (CLL) is characterized by almost ubiquitous CD71 expression (Smillevska et al., Leuk Res. 2006;30:183-9), this is in keeping with the activated status of CLL cells. In the present study, we evaluated TR1 and TR2 expression at the mRNA and protein level in 76 CLL patients as well as CD19+ B cells

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also evaluated, by real-time PCR, the expression of BCL2, ZAP-70, were measured by real-time PCR and semi-quantitative RT-PCR. We sorted peripheral blood (PB) mononuclear cells of two healthy donors. In all CLL cases, the tumor load (CD5+CD19+ cells) was at least 70%. Forty-eight out of 76 CLL cases (63%) carried IGHV genes with <98% homology to the closest germline gene (mutated); the remainder (28/76 cases, 37%) carried unmutated IGHV genes. TR1 and TR2 cDNA sequences were detected by RT-PCR in PB CD19+ B cells from both healthy donors. We found that both healthy donors expressed TR1 cDNA sequences; TR2-a/b cDNA sequences were detected by RT-PCR in, respectively, 39/76 and 70/76 CLL cases. Real-time PCR (using h-HPRT as a housekeeping gene) revealed comparable TR1 mRNA expression in CD19+ B cells of both healthy individuals (average TR1/h-HPRT ratio: 0.995) as well as higher TR2-a/b than TR2-a/b mRNA levels (average TR2-a/b/h-HPRT ratio: 0.07 and 0.016, respectively). TR1 mRNA levels were widely divergent in CLL samples (median TR1/h-HPRT ratio: 1.3 (0.87-13.5)). TR2-a/b mRNA levels were not significantly different in CLL vs. normal CD19+ B cells (median TR2-a/b/h-HPRT ratio: 0.07 (0.008-1.9)). In contrast, significantly higher TR2-a/b mRNA levels were observed in CLL vs. normal B cells [median TR2-a/b/h-HPRT ratio: 0.06 (0.005-0.23)]. In CLL, no statistically significant associations were identified between TR1 or TR2 mRNA levels and IGH mutation status. TR1 and TR2 protein expression was studied by Western blotting with appropriate antibodies. Quantitative gel-banding densitometry was conducted on Epson GT-8000 Laser Scanner. TR1 protein expression did not differ significantly in normal CD19+ cells vs. CLL (although CLL samples showed greater individual variation in TR1 band optical density). Faint TR2 bands were detected in normal CD19+ B cells (average optical density: 0.01), in marked contrast to CLL [median optical density: 0.4 (0.15-1.5)]. These results indicate that post-transcriptional mechanisms may play an important role in TR1 or TR2 expression in malignant CLL vs. normal B cells. TR2-a/b mRNA and TR2 protein expression are significantly more abundant in CLL, suggesting a possible functional role for TR2 in malignant cells, perhaps other than iron uptake. Finally, the results of the present study support the notion that the standard model of iron homeostasis, mediated mainly by TR1 and perhaps by TR2, remains the main mechanism to meet the demands for iron in either normal or CLL malignant B cells.

NEW PATHWAYS INVOLVED IN APOPTOSIS INHIBITION IN CHRONIC LYMPHOCYTIC LEUKEMIA
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Background. Apoptosis reduction mediated mainly by increased expression of BCL2 is a major mechanism of accumulation of mature B lymphocytes in peripheral blood (PB) and bone marrow in chronic lymphocytic leukemia (CLL). The disease comprises two subtypes that are characterized by important outcome differences observed between patients expressing either mutated (MUT) or unmutated (NAIVE) immunoglobulin heavy chain genes. We analyzed the gene expression profile of malignant cells of CLL (MUT and NAIVE) to identify possible additional molecular mechanisms related to decreased apoptosis. Materials and methods. CD19+ lymphocytes were isolated by positive magnetic cell sorting (MACS) from six CLL patients (3 MUT and 3 NAIVE), three mantle cell lymphomas (MCL) patients, and 4 normal naive B-lymphocytes isolated from tonsils. Gene expression profiles were obtained by cDNA microarray technology and correlated with clinical data of the patients.

In 22 patients with CD3+ LDGL, we investigated whether proliferating GL displayed the phenotype of fully differentiated cytotoxic cells. To this aim, according to the recently defined phenotypical pattern recognizing the different stages of cytotoxic effector cell differentiation, we carried out a comparative expression of CD27, CD56, CD45RA, CD45RO, CD3, CD69, CD8, CD28, CD44, CXCR4, CCR7, IFN-γ on GL surface. Since NK receptors have been found to be central in the pathogenesis of LDGL, we also analyzed the expression of Killer-Immunoglobulin-like Receptors (KIRs), CD94/NKG2, NKG2D and Natural Cytotoxicity Receptors (NCRs) on GLs surface. In all LDGL patients taken into account, GLs were found to be monotonically rearranged for the T-cell receptor (TCR). In 18/22 patients studied, our data showed that CD3+CD16+ cells expressed a homogeneous CD45RA+, CD27+, CD28+, CD62L+, CCR7-, IFN-γ+ pattern, consistent with those of fully differentiated CTLs. In four cases a coexpression of CD45RA and CD45RO was documented by GL. The majority of these patients (20/22) expressed NKGD2 receptor. In addition, KIR receptors were expressed only in a fraction of patients (7/22) as far as CD94/NKG2 (5/22). Interestingly, the activating receptor CD94/NKG2C was detected in 2/5 of CD94+ cases, suggesting that activating signals for cell proliferation might stem from this receptor. In all patients’ GLs the NCRs NKp44, NKp46, NKp80 were absent, while NKp80 was expressed in the majority of cases (20/22). In conclusion, our data demonstrated that GLs in CD3+ LDGL patients show a phenotype consistent with that of fully differentiated CTL. The expression of NK receptors, although useful for the definition of diagnosis of LDGL, does not represent a critical feature of the abnormal clone, suggesting that expression of these receptors is independent from the acquisition of the in vivo mature CTL phenotype, which indeed represents the truly distinctive phenotypic hallmark in these patients.

LOW-DOSE ALEMTUZUMAB MONOTHERAPY IN ADVANCED CHRONICLYMPHOCYTIC LEUKEMIA (CLL)
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Backgrounds. Alemtuzumab is a well-established therapeutic tool in CLL and its low-dose (ld) subcutaneous (sc) administration is safe and efficacious. Aim: To confirm on a larger number of patients with a longer follow-up the results of an already published (Cortezzelli A. et al Haematologica 2005; 90: 410-412) pilot study on ld sc alemtuzumab in refractory and resistant CLL patients. Methods. Since January 2003 we treated at our Institutions thirteen grade 3/4 infections were documented in one third of the patients. Previous grade 3/4 infections were documented in one third of the patients.
Alectumuzumab schedule was as follows: 10 mg sc X 3/ week for 18 weeks, with anti-infective prophylaxis. Results. The overall response rate (NClWG criteria) was 48.5%, including 21.2% complete response. Better responses were observed in blood (77.4%), as compared with bone marrow (54.5%), spleen (50%), and lymph nodes (48.5%). Responses were observed in patients with Zap70 (45%), adverse Karyotype (40%), 17p (60%), stage C (46%), fludarabine (28%) and rituximab-resistance. Progression was recorded in 7/15 patients after a median of 12 months (range 6-18 months). After a median follow-up of 15 months (range 1.5-28) the median survival has not reached for the entire case series (66.6% alive), responders (75%), and non-responders (58.8%). Eleven patients died after a median of 10 months (range 1.5-28) for infectious complications, 2 of them were in remission and 9 with advanced disease. Grade III-IV neutropenia or anemia were recorded in 36.1% and 2.7% of the patients, respectively. Mild anemia was observed in 55.5% of the patients during alectumuzumab therapy. Coombs-positive AIHA was documented in two patients after the end of Tx administration (9 and 1.5 months), being a reactivation in one of them. Two patients developed ITP one month after stopping alectumuzumab. Infections (101 cases media due to Pseudomonas, 1 dermatomycosis Herpes zoster, 2 pneumonia, 1 lethal polymicrobial sepsis) occurred during altemtumab treatment in 13.8% of the patients. Transient and clinically silent reactivation of cytomegalovirus was documented in 22.2% of the patients by pdd5 antigenemia testing or PCR. Eight patients (4 with latent and 4 with reactivated HBV infection) received lamivudine while on alectumuzumab. Adefovir dipivoxil was associated to lamivudine in two cases for an hepatitis flare. These antiviral therapies enables us to complete alectumuzumab Tx in all the patients. Conclusions. We confirm on a larger number of high-risk relapsing/refractory CLL patients the high percentage of response, long remission duration, and the favourable toxicity profile of ld sc Alectumuzumab already shown in the pivotal study.

ALLOGENIC STEM CELL TRANSPLANTATION AND CHRONIC LYMPHOCYTIC LEUKEMIA: DISTINCT IMMUNOGLOBULIN VARIABLE HEAVY CHAIN GENES AS A POSSIBLE PROGNOSTIC INDICATOR

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Backgrounds. Although the treatment of chronic lymphocytic leukemia (CLL) has changed dramatically over the previous few years, CLL remains an incurable disease. Great proportions of patients relapse early after the treatment and eventually become refractory to treatment. To a number of these subjects allogeneic stem cell transplantation (alloSCT) is being offered through the process of gene therapy which offers a different therapeutic approach and is associated with a number of risks. The mutational status of the immunoglobulin variable heavy chain (VH) genes is a major prognostic indicator for the clinical course and outcome of the CLL patients. More recently, additional prognostic categories have been identified by recognizing disease subsets which utilize unique VH genes. Experiments using,[1] V6-1, V8-2, V6-72 genes which are invariably associated with progressive and stable disease, respectively. Aims. The aim of our study was to investigate the possibility to identify suitable CLL candidates for alloSCT among CLL subsets that utilize unique VH genes. Methods. The study group consisted of 106 consecutive CLLs that had been diagnosing at our Institution prior to 2000, according to standard morphologic and immunophenotypic criteria. VH gene family usage and mutational status were obtained by direct sequencing of RT-PCR amplified RNA samples. Correlations between the different CLL subsets were made using standard statistical tests. Results. Our results showed that 61 (57.9%) patients utilize mutated VH genes, and the rest 45 (42.1%) have unmutated sequence. The most frequently rearranged VH gene in our CLLs was V6-169 gene, in all 25 cases (23.6%) the rest 45 (42.4%) have unmutated sequence. We compared the overall survival (OS) of the V6-169 subgroup against all the other patients. The two groups were comparable regarding the sex, age, total tumor mass (TTM) score and Rai stage. The VH-69 group has median OS of 56.7 months and all others patients have median OS of 125. 8, (p<0.0001). Then, we further analyzed the differences in survival between VH1-69 cases and all patients with unmutated VH genes. There were no differences between the two subgroups regarding the age, gender, TTM score, Rai stage and OS. No other unique VH gene, utilized in our study group, had frequency important of analyzing. Conclusion: We do not support the thesis that patients expressing V6-169 gene form a distinct subgroup of CLL patients. Further investigations are needed to reach the definite conclusion regarding the role of V6-169 gene and all others distinct VH genes in the prognosis and treatment decision in CLL patients. Our results confirmed that CLL with unmutated VH gene sequence has poorer OS and we suggest that all younger patients that utilize unmutated VH genes should be consider as candidates for early alloSCT, immediately after the first complete remission, if HLA identical donor is available.

REVERSAL BONE MARROW ANGIOGENESIS AFTER CONSOLIDATION THERAPY WITH ALEMTUZUMAB IN ADVANCED CLL

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We have previously shown that in patients with advanced chronic lymphocytic leukaemia (CLL) who respond to therapy with fludarabine bone marrow (BM) angiogenesis decreases significantly. To expand on these observations, we evaluated microvascular area of BM samples from 20 patients with advanced CLL (i.e., symptomatic Binet stage B or C) who received at least 8 weeks after the end of treatment with fludarabine subcutaneous alectumuzumab, three times weekly for 6 weeks, at escalating dose up to 10 mg. The patient sample included 14 males and 6 females with a median age of 51 years (range 44-60). After a median number of 6 cycles of fludarabine (range, 4-15), 11 (55%) patients could be classified in complete remission (CR) and 9 (45%) in partial remission (PR) (7 nodular-PR and 2 PR). Interestingly, the rate of CR increased to 90% (18 CR; P=0.03; Fisher’s exact test) after treatment with alectumuzumab. In keeping with hematological responses, significant changes of BM angiogenesis were observed. The assessment of microvesSEL area carried out at the starting of therapy, after fludarabine and at the end of therapy with alectumuzumab, respectively, showed a continuous decrease in the extent of microvesSEL area (p=0.002). This conspicuous feature was easily demonstrable in ZAP-70-positive (p=0.02) and ZAP-70-negative (p=0.001) patients. As far as molecular response is concerned, 13 out of 20 (65%) patients changed from a monocolonal to a polyclonal pattern of IgH. A separate evaluation carried out in patients with a persistent monoclonal IgH pattern and in patients who changed to a polyclonal pattern of IgH after therapy with alectumuzumab showed a significant reduction of BM microvesSEL area only in the latter (p=0.0002). Finally, a significant (p=0.0001) decrease of the extent of BM angiogenesis was observed among patients who received a cumulative dose of alectumuzumab higher than median (i.e., symptomatic Binet stage B or C) who did not apply for those who had received cumulative dose of alectumuzumab lower than median (p=0.127). In conclusion, a decrease in BM vascularity was observed after treatment with alectumuzumab. Such a finding reflects either molecular response or cumulative dose of alectumuzumab. These observations lend support to the anti-angiogenic role played by alectumuzumab in CLL.

FLUDARABINE AND CYCLOPHOSPHAMID (FC) VERSUS CYCLOPHOSPHAMID, VINCRISTINE AND PREDNISONE (CVP) AS FIRST LINE TREATMENT IN CHRONIC LYMPHOCYTIC LEUKAEMIA (CLL)

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Backgrounds. The combination of CVP is known to be active in previously untreated CLL patients. FC is another effective regimen. Aim. To evaluate the response rate, time to disease progression and survival of FC (arm A) versus CVP (arm B) as first line treatment. Patients and methods: The patients were randomized into the two treatment arms, 51 each arm. The diagnosis of CLL was established according to the criteria of the International Workshop on CLL (IW CLL) 1989. Eligibility criteria were age<65 years, ECOG performance status 0 -II with high risk category (Rai stage III-IV) or Rai stage I-II if they have at least one of the followings: one or more of the disease related symptoms, progressive marrow failure, massive splenomegaly or lymphadenopathy or progressive lymphocytosis > 50% in 2 months or lymphocyte doubling time < 6 months. Arm A: Cyclophosphamide 250 mg/m² I.V. D1 to D3, Fludarabine 25 mg/m² D1 to D3. Arm B: Cyclophosphamide 400 mg/m² D1 to D3, Vincristine 1.4 mg/m² D1 and Prednisone 100 mg/m² D1 to D5 P.O. Cycles to be repeated every 21 days. Hematological toxicity was recorded according to the NCI sponsored working
group revised guidelines for diagnosis and treatment of CLL (Cheson et al 1996). Evaluation of response was done according to the NCI-WG response criteria. Patient with stable or progressive disease after the 3rd cycle were excluded from the study while PR and CR cases continued to 6 cycles of the same regimen. Bone marrow biopsy and Immunophenotyping were done to confirm the response to treatment. Results. The median age of the whole group was 69 years (Range 33 - 86). They included 42 males and 20 females. Twenty cases had stage III and 21 patients for each of stage II and III. The median TLC was 81×10^9/L. The median lymphocyte count was 70×10^9/L. The median hemoglobin level was 9.2 gm/dl, while the median platelets count was 150×10^9/L. Pre-treatment bone biopsy showed diffuse pattern in 49 cases (79%) and the treatment bone biopsy showed diffuse pattern in 49 cases (79%) and the treatment bone biopsy showed diffuse pattern in 49 cases (79%) and the treatment bone biopsy showed diffuse pattern in 49 cases (79%) and the treatment bone biopsy showed diffuse pattern in 49 cases (79%). Twenty cases had stage III and 21 cases in arm A (32.3%) and only 3 cases in arm B (9.7%). Partial response with nodules (PR-nod.) was reported in 5 cases (16.1%) in arm A and 3 cases (9.7%) in arm B. Median time to disease progression was 25 months in arm A and 6 months in arm B (p=0.03). At 2 years, no significant difference in survival between both arms was detected (83.9% for arm A versus 74.2% for arm B). Conclusion. The combination of FC is able to induce higher response rate with better quality of response at the level of BM biopsy. There was a statistically significant difference in time to disease progression in favor of FC regimen, but no significant difference in overall survival.

0792 ORAL FLUDARABINE AND CYCLOPHOSPHAMIDE IN UNTREATED PATIENTS AFFECTED BY CHRONIC LYMPHOCYTIC LEUKEMIA. A PILOT STUDY

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Backgrounds. The introduction of fludarabine into the treatment of chronic lymphocytic leukemia (CLL) has improved the complete remission rate (CR), overall response rate (OR), and progression free survival (PFS) compared with alkylator based regimens. Its synergistic action with cyclophosphamide as front-line therapy in untreated CLL patients with advanced disease. The oral formulation of fludarabine showed a similar safety profile and response rate as the front-line approach, at the end of the compound. Aims. Primary end-point was to test efficacy and safety of the oral formulation of fludarabine combined with cyclophosphamide as front-line therapy of high-risk CLL. As secondary end-point we examined the impact of new prognostic factors associated with progressive CLL (i.e., unmutated IgVH gene status, positivity for ZAP-70, del(11)(q23), and del(13)(q14) on mononuclear cells. CLL patients did not receive specific therapy till the moment of cytogenetic analysis. Results. Del13q14 was the most frequent cytogenetic abnormality; it was revealed in 34 (25%) cases. Del11q23 was found in 26 (19%) cases, trisomy 12 in 17 (13%) cases and del1p13 in 8 (6%) cases. Complex karyotype was obtained in 8 (6%) patients. B-CLL was diagnosed in 11 (8%) patients, del13q21 (73%) of them received first line therapy consisting of alkylator based regimens. Of the remaining 21 patients 3 had stable disease and 3 progressive disease. In terms of haematological toxicity 6 patients developed grade IV neutropenia and received G-CSF treatment, while two patients developed severe anemia (grade III and IV) that required red blood cell transfusions. Only one patient developed a transient febrile neutropenia of unknown origin, but did not require hospitalization. Mild extra-hematological toxicity consisting of nausea and vomiting occurred in six patients during the treatment. No significant differences were noticed in terms of CR and OR rate between the IgVH mutated and unmutated groups (p=ns). Among the 3 patients who have relapsed so far, 4 had unmutated and only 1 had mutated IgVH genes (p=ns), and all three patients that required new treatment (NCI WG criteria) had unmutated IgVH. Conclusion: Oral fludarabine plus cyclophosphamide as front-line therapy in CLL achieved a good overall response rate in our series of patients (46% CR and 31% PR). The haematological and extra-hematological side effects were mild and the oral formulation was easy to administer. The differences in terms of CR, OR and PFS between the IgVH mutated and unmutated groups did not reach statistical significance. However, a longer follow-up is required to define the possible correlation between these prognostic factors and treatment outcome.
IGVH MUTATIONAL STATUS, VH GENE USAGE, GENDER AND PROGNOSIS IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Backgrounds. Somatic hypermutation in the variable region of the immunoglobulin heavy chain gene (IGVH) has been shown to be a powerful prognostic parameter in chronic lymphocytic leukemia (CLL) and has the capacity to differentiate between two disease subsets. In recent years individual IGVH gene rearrangements, in particular VH3-21, have been shown to define unique disease entities in CLL. Aims. To determine the most commonly expressed VH genes in a Northern Irish cohort of CLL patients and to ascertain if variations in gender, light chain restriction and the presence of chromosomal abnormalities show associations with IGVH mutational subgroups and individual gene rearrangements.

Methods. Two hundred and twenty-eight CLL patients were recruited from Belfast City Hospital and surrounding hospitals. IGVH mutational status and VH gene usage were determined using standard BIOMED-2 primers and protocol followed by sequence analysis. FISH analysis was performed to identify the presence of recurrent chromosomal aberrations. Light chain restriction was determined by standard immunophenotyping techniques. Results. The most common VH gene rearrangements were VH4-34 (13.5%), VH1-69 (12.3%), VH1-2 (7.9%), VH3-21 (7.9%) and VH1-3 (7.5%). Females showed a bias towards mutated IGVH status (2M: 1UM), overuse of VH4 genes (40%) and a lower frequency of trisomy 12 (7%). In contrast males showed no mutational bias (1:1), overrepresentation of VH1 genes (35%) and a higher incidence of trisomy 12 (21%). VH3-21, VH3-48 and VH3-53 showed preferential lambda light chain restriction, while VH1-69 had overrepresentation of kappa light chains. Further analysis of VH3-48 and VH3-53 gene usage showed a preponderance of females (7:1; 3:1 respectively), lambda light chain restriction and inferior outcome irrespective of IGVH mutational status. Conclusions. This study has demonstrated gender related differences in CLL, which can be explained partly by the increased incidence of mutated IGVH genes, biased use of VH4 genes and lower frequency of adverse chromosomal aberrations in female patients. This study confirms that gender related survival differences exist in CLL patients and that gender should be taken into account in risk stratification of patients at presentation. Furthermore, specific VH gene usage is associated with have distinctive characteristics, supporting the concept that antigens have an important role to play in the aetiology of CLL.

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Backgrounds. Currently immunoglobulin heavy chain variable region (VH) gene mutation status considered to be the most accurate discriminator of clinical outcome in CLL. However, the latest data suggest that individual VH genes might also have prognostic significance, independently of mutation status. Aim. The aim was to refine the significance of VH mutation status and VH gene usage for prognosis of CLL course. Methods. Total RNA was extracted by guanidinium thiocyanate method from peripheral blood lymphocytes and reverse transcribed into cDNA. VDJ sequences were amplified using VH-FR1/JH primers as recommended by BIOMED-2 protocol. Puriﬁed PCR products were sequenced directly with automated DNA sequencer and sequences were analyzed using international databases (IMGT, IgBlast, V-BASE). Results. We sequenced VDJ rearrangements in 76 CLL patients from different regions of Ukraine. Of the 51 functional VH genes, 24 were identiﬁed, which represented 3 VH gene families - VH3 (64; 44.7%), VH1 (28; 36.8%), and VH4 (14; 18.4%). The most frequent VH gene was VH1-69 (19 cases, 25%) followed by VH4-34 and VH3-33 (5 cases each; 6.6%), VH3-07, VH3-30, VH3-09 and VH3-21 were used in 4 cases each (5.3%). Unmutated were 53 cases (69.7%) and 23 cases (30.3%) were considered as mutated ones using 2% mutation border. All VH-69 cases had a germinal conﬁguration, and this gene was the most frequently represented VH gene in the unmutated subset. Within the mutated subset the most frequent gene was VH3-07. In comparison with mutated cases, patients with unmutated VH status had shorter median time to progression (40 months vs. 91 months; Log Rank 7.24; p=0.0071), more frequent marked lymphadenopathy and splenomegaly at the moment of diagnosis (13/53 vs. 0/23; p=0.008), though differences in total survival were non-signiﬁcant (median 49 months for mutated cases and did not reach for unmutated cases; Log Rank 2.53; p=0.1115) due to a short time of observation. All patients resistant to therapy with progressive disease according to NCI-WG belonged to unmutated cases from VH1 (6) and VH3 family (6) (p=0.02). Autoimmune disorders were registered mainly among unmutated VH1 (8-VH1-69, 2-VH1-58, and 1-VH1-45) and VH3 cases (1-VH3-09, 1-VH3-11, and 1-VH3-21) and only one patient had mutated VH1-02 gene. Secondary cancer has developed in 2 patients with unmutated VH1-69 (stomach, urethra) and in one patient with mutated VH3-07 (skin). On the contrary, Richter transformation was observed only in patients with VH3 and VH4 genes (VH3-01 and VH3-48 mutated; VH3-09, VH3-20, VH3-33, VH3-53, and VH4-34 unmutated) (p<0.05 in comparison with VH1 gene family). The worth course of disease had 2 unmutated VH3-09 patients (dead, survival 11 and 49 months), and the best-the patient with mutated VH3-21 gene with short CDR3 sequence - ARDMNAMDV (alive, survival 247 months, in complete remission after fludarabine treatment, duration 26 months).

Conclusion: While the significance of VH mutation status for prognosis of CLL course is beyond question, some features of the disease might be associated with individual VH gene usage.
Efficacy of Rituximab in Hairy Cell Leukemia

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Backgrounds. Hairy cell leukemia (HCL) is a rare B-chronic lymphoproliferative disorder with an indolent course. First-line treatment modalities include 2-chlorodeoxyadenosine, 2-deoxycoformycin and interferon-α. The efficacy of anti-CD20 (Rituximab) in other BCLDs and the strong expression of CD20 by hairy cell leukemia lymphocytes indicate that Rituximab could be an alternative in the treatment of HCL. The experience of a single Hematology Unit in the treatment of relapsed HCL with Rituximab. PATIENTS AND METHODS. We retrospectively analyzed all hairy cell leukemia patients who received Rituximab as salvage therapy in 1st or subsequent relapse. The clinical and laboratory characteristics at diagnosis and relapse, primary treatment and its duration, as well as response to treatment were analyzed. Results. 10 patients treated with Rituximab were located among 110 patients diagnosed with HCL between 1980 and 2005. 9 were males and their median age at diagnosis was 46 years (range: 42-94). All but two patients presented with splenomegaly with a median spleen size of 8.5 cm below left costal margin (range: 2-30 cm). 9/10 patients were leukaemic at diagnosis with the remaining one showing lymphocytosis. All patients displayed a typical immunophenotype from blood and/or bone marrow (CD20 strongly+, CD19-, CD22+, FMC-7+, CD11c+, CD25+, CD103+). Six patients received Rituximab at 1st relapse. Among them, one had received 2-deoxycoformycin as 1st line treatment, one 2-chlorodeoxyadenosine and four interferon-α as induction and maintenance. 3 patients had received more than one prior treatments. Two of them were IFN-α resistant and one had discontinued IFN-α due to side-effects. One patient received Rituximab at diagnosis, due to older age and possible complications with other therapies. The median time from diagnosis to Rituximab initiation for the 9 patients was 61 months (range: 19-168). Rituximab was administered at 375 mg/m² weekly for 6 cycles. Overall response rate was 77%. 2 patients went into complete remission, including a negative bone marrow biopsy, a negative immunophenotype and a negative immunoglobulin gene rearrangement. 2 patients achieved a complete hematologic remission with normalization of their cytopenias, but with remaining bone marrow infiltration of <25%. 3 patients achieved a partial hematologic response, while 1 patient was Rituximab resistant. One patient is not evaluable and the remaining one discontinued treatment after the first cycle due to the development of thrombocytopenia that was attributed to the drug. No other complications were recorded, except of mild infusion-related symptoms. Among the complete hematologic responders, no patient has relapsed with a median follow-up of 12 months (range: 4-38). Among partial responders, one achieved a complete response including a negative bone marrow and immunophenotype after Rituximab retreatment, one is alive in partial remission without the need of further therapy and one progressed within 5 months from Rituximab administration. Conclusions. Rituximab is a highly effective treatment for HCL in relapse with a response rate of 77%. Retreatment with Rituximab, or maintenance may be important, since ongoing responses are seen.

Successful and Cost-Effective Prophylaxis and Treatment of Tumor Lysis Syndrome (TLS) with Low Doses of Rasburicase

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Backgrounds. TLS commonly occurs in patients with hematologic malignancies and is characterized by elevation of uric acid, potassium and phosphate and by hypocalcemia. A major complication is renal failure caused by precipitation of uric acid and/or calcium phosphate crystals. Treatment consists of hydration, correction of electrolyte disturbances and lowering of uric acid. Rasburicase, a recombinant urate oxidase, has proven to be highly effective in lowering serum uric acid levels, and its application is not restricted in patients with renal failure. Costs for a full seven-day course of rasburicase in the dosage recommended are high and amount to approximately 4800 €. For rasburicase a 96% reduction of uric acid levels 4 hours after the first dose has been reported. Therefore, the question arose whether lower doses of rasburicase than those recommended by the manufacturer can be applied for efficient prophylaxis and treatment of TLS. Patients and Methods. 40 patients (27 male, median age 65 yrs, range 16-88) received rasburicase in low doses for prophylaxis or treatment of TLS. Ten patients had acute leukemia, 6 had myeloproliferative disorders, 5 patients had high-grade Non-Hodgkin’s lymphoma, 15 had low-grade Non-Hodgkin’s lymphoma and 4 patients had solid tumors. TLS was classified into laboratory and clinical TLS and graded according to Cairo and Bishop. Thirty-five patients had elevated serum creatinine levels, the median creatinine level in these patients was 2.63 mg/dl (1.18-8.61 mg/dl). Laboratory TLS was diagnosed in 5 patients, 28 patients had clinical TLS. Results. Seven patients received rasburicase for prophylaxis of TLS. The mean LDH level in this group was 706 U/l, the mean uric acid level was 11.7 mg/dl before application of rasburicase. The median number of doses of rasburicase applied was 2 (range 1-2), the median total dose was 3 mg (0.052 mg/kg). After application of rasburicase the mean uric acid level decreased by 66% and was 3.7 mg/dl. None of the patients developed TLS. Thirty-three patients received rasburicase for treatment of TLS. The mean LDH level was 1668 U/l. The mean uric acid level was 15.8 mg/dl before application of rasburicase. The median number of doses of rasburicase applied was 1 (range 1-5), the median total dose was 3 mg (0.098 mg/kg). After application of rasburicase the mean uric acid level decreased by 80% and was 3.2 mg/dl. No patient required renal replacement therapy. Rasburicase was well tolerated by all patients without side effects. Conclusion. We applied rasburicase doses as low as 3.2-4.3% of the dose recommended by the manufacturer. Rasburicase applied in low doses proved to be effective for prophylaxis and treatment of TLS, even in patients with renal failure. For some patients doses as low as one vial of 1.5 mg of rasburicase was sufficient to control hyperuricemia, lowering the costs to 83. Cost-effective treatment becomes an increasingly important issue regarding limited budgets in health care. Further studies have to be conducted to establish dosing regimens for different clinical settings.
ABSTRACT WITHDRAWN

IMPROVEMENT IN HEMOGLOBIN LEVELS AND QUALITY OF LIFE IN ANEMIC PATIENTS WITH HEMATOLOGIC MALIGNANCIES RECEIVING EPOETIN ALFA DURING CHEMOTHERAPY: RESULTS FROM THE EPOLYM TRIAL

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Backgrounds. Anemia from disease and/or chemotherapy is a common complication in patients with Hodgkin’s disease (HD), non-Hodgkin’s lymphoma (NHL), chronic lymphocytic leukemia (CLL), and multiple myeloma (MM). Because anemia is associated with fatigue and other symptoms of diminished quality of life (QOL), treatment of anemia in patients with hematologic malignancies is important. Aims. The primary objective was to compare change in QOL from baseline to week 12 when anemia is corrected with a once-weekly (QW) regimen of epoetin alfa in patients with HD, NHL, CLL, or MM receiving chemotherapy. Improvements in Hb levels and transfusion requirements were also recorded. Methods. EPOLYM was an international, multicenter, open-label, Phase IIIb, 24-week trial in anemic (hemoglobin [Hb] ≤12 g/dL) cancer patients receiving chemotherapy. Patients received epoetin alfa 40,000 IU QW subcutaneously to a target Hb of 11.5-13.0 g/dL with dosage adjustments based on clinical response. The primary objective of QOL improvement was evaluated by the Functional Assessment of Cancer Therapy-Anemia (FACT-An) which measured functional well-being (FWB), fatigue subscale score (FATS), non-fatigue subscale score (NFATS), and total anemia subscale score (ANS). The Linear Analog Scale Assessment (LASA; also known as the Cancer Linear Analog Scale (CLAS)) for energy level, daily activities, and overall QOL was also employed. Statistical significance was analyzed by the paired sample t-test. Changes in Hb level and transfusion requirements from baseline were evaluated. Results. Intent-to-treat population was 1084 patients: 416 NHL, 307 MM, 165 HD, and 145 CLL (1 unspecified). Epoetin alfa dosage was increased for 57.5% of patients. Mean baseline FACT-An scores were: FWB 14.9; FATS 31.3; NFATS 19.4; ANS 50.7. Mean increases for FACT-An from baseline to week 12 and baseline to week 24 were significant for all parameters: FWB (p<0.005); FATS (p<0.001); NFATS (p<0.001); ANS (p<0.001). Mean baseline LASA scores ranged from 46.5 to 50.4 (normal: 70 to 100) indicating poor QOL at study initiation. Mean changes in LASA at week 12 were clinically* and statistically significant: energy level, 8.9 mm (p<0.001); daily activities, 7.5 mm (p<0.001); overall QOL, 6.8 mm (p<0.001); improvements were even greater at 24 weeks for all parameters. Both FACT-An and LASA scores improved as Hb level increased, with increases ≥2 g/dl demonstrating the greatest mean improvements at week 12 and 24. Baseline mean Hb for combined diagnostic groups was 10.4 g/dL; patients with MM had the lowest mean Hb (10.0 g/dL) and patients with HD had the highest mean Hb (10.8 g/dL). Mean Hb increased after 3-5, 12, and 24 weeks of epoetin alfa treatment were 1.0 g/dL, 1.7 g/dL, and 1.7 g/dL, respectively (p<0.001 at all time points). MM patients had the greatest Hb increase at week 12 (2.1 g/dL). Transfusions were administered to 25% of patients during the study. Conclusions. Treatment with epoetin alfa resulted in improved QOL in anemic patients with HD, NHL, CLL, and MM. This improvement was associated with increases in Hb attained with epoetin alfa 40,000 IU QW.


COST AND COST-EFFECTIVENESS OF FLUCAM VERSUS FCR IN PATIENTS WITH RELAPSED OR REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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Backgrounds. The most promising approaches for treatment of relapsed CLL involve the use of drug combinations with complimentary mechanisms of action against the disease, and immunotherapy combinations have proven to be particularly effective. However, these combination therapies may also be associated with higher costs, and in any treatment decision cost needs to be weighed against the added therapeutic benefit. Aim. This analysis compares the likely costs and expected health benefits of the emerging fludarabine + alemtuzumab (FluCam) combination versus the fludarabine, cyclophosphamide, and rituximab (FCR) regimen in relapsed/refractory CLL patients. Methods. The effectiveness evidence used for analysis was derived from 2 published phase II clinical studies evaluating FluCam or FCR in the relapsed CLL setting: Elter et al. 2005 and Wierda et al. 2005. Overall response rates (ORR) and expected months in remission per patient treated were outcome measures used to assess the effectiveness or health benefit of each drug combination; the latter was estimated by multiplying a weighted ORR by the duration of response. Resource use associated with each intervention was based on published literature, a previous patient level costing study conducted in the Netherlands in follicular lymphoma, and expert opinion. Unit costs for the Netherlands were derived from local hospital accounting systems, published tariffs, and wholesale prices listed for...
2003, the price year chosen for this analysis. Sensitivity analyses were conducted to test the robustness of the assumptions. Results: The mean total cost of FluCam and FCR treatment was estimated at approximately €26,426 and €35,324, respectively, assuming 6 cycles of therapy. The ORR reported for FluCam is 85% and for FCR it is 73%, which would yield a cost per responder of €61,838 and €48,389, respectively. Data on response duration for either therapy are currently not available, but pretreatment and time to progression (TTP) are 15 months for all patients and 28 months for responders. Assuming as a base-case scenario a 73% ORR and 24 months of response duration for each therapy, then the expected number of months in remission per patient treated would be 17.52 months, or 1.46 years. Comparing costs and assuming a similar level of clinical benefit, the cost per patient in remission for FluCam and for FCR would be €18,100 and €24,194, respectively. Sensitivity analyses suggest that FCR would need to be at least 25% more effective than FluCam to achieve equivalence in terms of cost-effectiveness, which is unlikely given that alitretinoin is more effective than rituximab as a monotherapy. Conclusions: Based on this analysis, FluCam is potentially a more cost-effective treatment strategy for relapsed refractory CLL. The findings of this analysis imply that for each relapsed CLL treatment where FluCam is used rather than FCR, the cost savings to the payer would be on average €8,898 if both therapies were equivalent in terms of efficacy, and even more if FluCam is the more effective alternative. Randomized trials comparing the effectiveness and cost of both combinations are needed to confirm the findings of this analysis.

0802

PAIN IN HAEMATOLOGY: AN OUTCOME RESEARCH PROJECT TO EVALUATE THE EPIDEMIOLOGY, PATHOPHYSIOLOGY AND EFFECTS OF PAIN TREATMENT IN IN-HOSPITAL PATIENTS

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Background: Management of pain associated with blood-related diseases is often difficult and inadequate, notwithstanding the availability of several therapeutic interventions and of well-known guidelines and protocols. Patients and Methods: A six months multicenter study involving the wards of three Italian haematological Centres was started in October 2005 to investigate the epidemiology and the clinical characterisation of pain in haematological patients. A treatment protocol based on the cancer pain WHO analgesic ladder was applied. Data reported as January 2006 included 266 patients (166 male) with a median age of 67 (13-89) years. The total number of admissions during the study period was 415. Haematological diagnoses were as following: 85 (80%) non-Hodgkin’s lymphomas, 55 (20%) acute myeloblastic leukaemia (AML), 29 (10%) multiple myeloma (MM), 16 (6%) acute lymphoblastic leukaemia (ALL), 29 (10%) other lymphoproliferative disorders, 26 (9%) myelodysplastic and chronic myeloproliferative disorders and 46 (16%) non-malignant diseases. Results: Of 286 patients, 128 (45%) experienced almost one pain syndromes, for a total of 174, which pathophysiology were diagnosed as follows: 76 (43.6%) deep somatic, deriving from the bone in most cases, 34 (19.5%) superficial somatic (cutaneous and cutaneous) 30 (17.2%) visceral, 15 (8.6%) neuropathic, 15 (8.6%) mixed or by unknown mechanisms and 4 (2.2%) iatrogenic or infection related. A diagnosis of MM, ALL and AML, and an advanced disease phase were significantly associated with a higher incidence of pain. The treatment protocol was based on three steps, according to the intensity of pain. The first step (mild pain) included paracetamol, the second step (moderate pain) tramadol, and the third the step (severe pain) morphine. In selected cases, oxycodone or fentanyl patches were used in the place of morphine to treat severe pain. Of 174 pain syndromes, 48 (28%), 49 (29%) and 77 (53%) pain syndromes were treated with a first-line therapy according to the first, the second and the third step respectively. Of the 97 pain syndromes treated with the first two steps, 57 (58%) needed a treatment escalation to the third step after 4 (1-12) days. No serious adverse effects were recorded. The adopted treatment approach, integrating causal interventions (if applicable) and analgesic measures, including the adjuvants to treat neuropathic pain, allowed a prompt relief in more than 90% of the pain syndromes. No serious adverse effects were recorded. Conclusion: These preliminary results indicate that, in the setting of haematological wards, pain is a significant symptom requiring prompt medical attention. Moreover, our data outline that most pain syndromes can be effectively controlled by the currently available treatment strategies. Therefore, the implementation of clinical pathways and standardized protocols based on well-defined algorithms can provide the auspicial advancements toward a ‘pain-free’ haematological hospital.

0803

COST-EFFECTIVENESS OF ‘Y-IBRITUMOMAB TIUXETAN (‘Y-ZEVALIN) VERSUS RITUXIMAB MONOTHERAPY IN PATIENTS WITH RELAPSED FOLLICULAR LYMPHOMA

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Backgrounds: Currently, there are limited cost-effectiveness data comparing the use of 90Y-Zevalin with rituximab in follicular lymphoma (FL). Aims: The objective of this analysis was to estimate the (incremental) cost-effectiveness of a single dose of 90Y-Zevalin 0.4 mCi/kg compared with: 1) standard rituximab treatment of 375 mg/m2 weekly for 4 weeks (4-dose rituximab); and 2) standard rituximab followed by 4 weeks of maintenance therapy (8-dose rituximab) in patients with FL. Methods: The cost-effectiveness data used for the analysis were derived from the only 2 clinical studies published to date enrolling comparable populations where patients had received either ‘Y-Zevalin or rituximab monothera-
Cost-effective strategy. 

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The results of which were published by Gordon et al. (2005), and, for the 8-dose rituximab arm, a study published by Chiehminni et al. (2004). Expected months in remission were used as the measure of effectiveness for the analysis. Months in remission were estimated by multiplying the overall response rates for each therapy by the response duration. To estimate the resources involved in the management of adverse events, we consulted a panel of experts. All other resource use estimates, ie, those for administration, prophylaxis, and monitoring, were derived from clinical guidelines and the randomized trial database. Unit costs for Netherlands were derived from local hospital accounting systems, tariffs, and listed wholesale prices for medication. The price year was 2005 for all costs except 90Y-Zevalin, for which it was 2006. Results. The mean total cost of treatment with 90Y-Zevalin was estimated to be approximately EUR 18,274. The mean total cost of treatment with 4 doses of rituximab was estimated to be lower at EUR 9,847, whereas the cost associated with an 8-dose rituximab treatment was EUR 20,112. In terms of health benefits, the average number of disease-free months per patient treated was highest for 90Y-Zevalin at 14.4 months followed by 11.4 months for the 8-dose rituximab and 6.2 months for the 4-dose rituximab. When the estimates of health benefit are combined with costs, the analysis demonstrates a mean cost per disease-free month for 90Y-Zevalin of EUR 1,272, the lowest of the 3 therapies, followed by EUR 1,599 for 4-dose rituximab therapy, and EUR 1,770 for 8-dose rituximab. Conclusions. The findings imply that for each third-line follicular NHL treatment where 90Y-Zevalin is used rather than 4-dose rituximab, the additional cost to the payer would be, on average, EUR 8,426. For this additional cost, the benefit to the patient would be an average 8.2 additional months in remission, over and above what would have been gained with 4-dose rituximab therapy. Furthermore, when the costs and benefits of 90Y-Zevalin are compared with the 8-dose rituximab regimen, 90Y-Zevalin is the more cost-effective strategy.

**0805**

**COST EFFECTIVENESS OF ADDING IMATINIB TO CHEMOTHERAPY IN ADULT PATIENTS WITH PHILADELPHIA CHROMOSOME-POSITIVE ACUTE LYMPHOBластIC LEUKEMIA: AN EXPLORATORY ANALYSIS**

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Background. The prognosis of Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ALL) in adults is extremely poor. Imatinib has been reported to successfully induce and consolidate remissions and extend disease-free survival in Ph+ ALL patients with good tolerability in clinical studies. Aims. This study explores the cost effectiveness of imatinib plus conventional chemotherapy (CC) vs CC alone in adult Ph+ ALL patients. Methods. A Markov model was used to follow a hypothetical cohort of 1000 adult Ph+ALL patients receiving imatinib plus conventional chemotherapy (CC) or CC alone. Patients were modeled for a total of ten years in monthly intervals. Patients were distributed over time into three health states: alive without disease progression (DFS), alive with disease progression (DS), or dead. For the purpose of this model, patients were assumed to continue imatinib therapy (600 mg daily) for up to two years as long as they remained in the DFS state. Probabilities of being in the states were derived from the published literature of case series for patients with Ph+ALL receiving either treatment. In the absence of relevant data pertaining to Ph+ALL, assumptions about costs and utilities were derived from a cost analysis of chronic myeloid leukemia. Only direct medical costs were included in the analysis using a U.S. health care payer perspective. All outcomes were discounted at a 3% rate per annum. Results. Based on the literature, the median disease free survival and overall survival of Ph+ALL adult patients with CC were 6 and 9 months (Thomas X et al. 1998), respectively. The 12-month disease free survival and survival for imatinib+CC were 72% and 84% (Towatari 2004), respectively. Total discounted survival was 1.02 years for CC and 4.29 years for imatinib+CC. Total discounted disease free survival was 0.76 year for CC and 2.79 years for imatinib+CC. Assuming utility weights of 0.85 and 0.85 for DFS and DS, respectively, the total discounted quality adjusted life years (QALY) were estimated to be 0.85 vs. 0.83 for CC and imatinib+CC, respectively. Thus, the net incremental gain in quality adjusted survival was 2.47 QALYs. Total incremental treatment costs for imatinib+CC were $102,507 as compared to CC over 10 years. Therefore, the incremental cost per QALY of imatinib+CC vs CC alone was approximately $41,500 (i.e., $102,507 divided by 2.47 QALYs) which is within the range of usual acceptable cost effectiveness threshold. Conclusions. For adult ALL patients with poor prognosis due to Ph+ALL, our exploratory analysis suggests that, given the underlying data and assumptions, adding imatinib to current chemotherapy regimens may be cost-effective compared to chemotherapy alone.

**0806**

**MASS NEONATAL CORD BLOOD SCREENING: COST-EFFECTIVE IDENTIFICATION OF HEMOGLOBINOPATHIES**


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Backgrounds. The diagnosis of haemoglobinopathies is of growing importance particularly in countries with high incidence of disorders of globin chain synthesis, both thalassemias and structurally abnormal hemoglobin. Sickle cell disease is a major public health problem in the Sultanate of Oman with a high rate of morbidity and mortality. The
overall prevalence of Sickle cell trait of 6% (2.9-10%) whereas that of thalassemia trait is 2-3%. Aims: To ascertain the feasibility of neonatal cord blood screening in the Sultanate of Oman in an effort to determine the prevalence of haemoglobinopathies by a cost-effective method. Methods. A total of 1575 cord blood samples were screened for presence of possible haemoglobinopathies by high performance liquid chromatography (HPLC) technique using Biorad Variant™ program between April 2005 & February 2006. Complete blood counts were also obtained on Cell Dyn 4000 automated blood cell counter. All samples were then processed to isolate and store mononuclear leukocytes for subsequent molecular diagnostics. Results. The findings indicated a 37.21% incidence of α-thalassaemia (predominantly -α-7.7αα). Furthermore, the overall incidence of other haemoglobinopathies was 10.38% with 6.45% and 2.36% incidence of sickle haemoglobin and β-thalassaemia respectively. On HPLC, D-window, E-window and C-window were present in a small group of neonates that require further testing. Moreover, the cost of blood screening cannot give a definitive diagnosis it can identify the strategy towards prevention of haemoglobinopathies. Although cord blood screening cannot give a definitive diagnosis it can identify the small group of neonates that require further testing. Moreover, the cost of testing per sample was approximately 1 Euro.

Figure 1. Prevalence of haemoglobinopathies in the newborn.

0808
VALUE OF TRANSFUSION-FREE LIVING IN MDS: RESULTS OF HEALTH UTILITY INTERVIEWS WITH PATIENTS
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Backgrounds. Achieving transfusion independence in patients with transfusion-dependent MDS has been defined as a key treatment goal in clinical trials of new interventions and in everyday clinical practice as new treatments have become available. However, data is lacking on how patients themselves rate their health status in relation to transfusion independence as opposed to transfusion-dependence. Methods. We performed health utility interviews with MDS patients in the US, France, and the UK to elicit the value of transfusion-independence or reduced transfusion burden compared to transfusion dependence (i.e., three distinct health states). Health state descriptions were developed based on literature and reports from MDS patient focus group discussions, and were validated by a haematologist. Each health state card included different severity/intensity of problems on the following quality-of-life (QoL) domains: reliance on blood transfusions and health care provider facility; need to arrange one’s life around medical appointments; fatigue and tiredness that limits performance of routine physical activities; interference of disease with social and family life; worry about the future due to health condition; discomfort associated with medical conditions and treatment, and the feeling of being at risk of infection; reliance on support persons for self care and routine activities; feelings of being a burden to family; and feeling sad, hopeless, and helpless. Face-to-face interviews used the Feeling Thermometer visual analogue scale (F-TTO) and the Time Trade-off (TTO) method to value the health states on a scale anchored on 1 (perfect health) and 0 (dead). We administered background questionnaires on socio-demographic, clinical, and QoL (EQ-5D) characteristics to describe the patient sample. Results. Thirty-eight MDS patients in the US (n=8), France (n=9), and UK (n=21) completed the interview. The mean age was 66 years (range: 29-83), 55% were male. The majority were retired (66%), had secondary/high school education (38%) or higher (24%), and were living with family, a partner or spouse, or friends (76%). The mean time from MDS diagnosis was 5 years (range: 1-25). The majority of patients received blood transfusion(s) previously (87%), and 47% had received a blood transfusion in the last three months. Mean EQ-5D utility score was 0.78, and patients reported at least some problem with mobility (44%), usual activities (39%), pain/discomfort (47%), and anxiety/depression (29%). One patient reported problems with self-care. Few patients had difficulty understanding the rating scale (n=3) and TTO (n=5) exercise. The health utility score for the transfusion-independent health state was significantly higher than for health states with reduced transfusion requirement (0.85 vs. 0.77, p<0.001), and transfusion dependence (0.85 vs. 0.62; p<0.001). Three patients valued transfusion dependence as worse than being dead. Corresponding rating scale scores were 78 vs. 57 (p<0.001) and 78 vs. 32 (p<0.001), respectively. Conclusions. These results show that patients associate a high value with achieving transfusion independence, which, in turn, suggests an important role for new treatments aiming to achieve greater transfusion independence in MDS.

0807
FOLLOWING THE CASE OF ESSENTIAL THROMBOCITOSIS CAUSED BY NOURISHING DEFICIENCY
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Laboratory. Claudio Mallem & Associated, GENERAL ALVEAR, Argentina

Thirty two male longevous patients, residents in an old people’s home and abandoned by their relatives, were studied. At the start of this case-study the patients had their blood values, which showed severe anaemia and microcytosis accompanied with trombocitosis, badly altered. It was decided to move the old men to another home, where the nourishing diet was radically changed, leaving out the former one which consisted of carbohydrates the seven days of the week and changing it for the ingestion of protein, vegetables and diary products nutrients. During the first forty five days they were helped with ferrous anhidro succinate to raise the level of hemoglobin, whose value was between seven and eight g/dl (in average). At the next blood control it was clear-sighted that slowly and gradually the values were encouragingly changing, that’s why it was decided to suspend the ferrous anhidro succinate and allow the new diet to perform the corresponding supply. After 90 days, at the corresponding blood control, it was shown that the patients had got normal analytical values, recovering their health, vitality and basically their right to enjoy completely a better quality of life, thus achieving psycho-social stability.
or MDS, currently undergoing infusion ICT, completed the SF-36 and CHQ. In total, 79 participants were assessed (US: thalassemia n = 41 and SCD n = 9; UK: thalassemia n = 11, SCD n = 14, and MDS n = 4). The HRQoL instruments, the SF-36 and CHQ, were scored (0 to 100, with higher scores indicating higher QoL) and compared to available age- and/or gender-matched published norms for UK and US. In addition, utilities were estimated for the UK population using published algorithms to transform SF-36 scores into utility-based scores. Results. In the US, compared to age- and matched norms, study participants scored lower on all SF-36 domains (decremental point difference ranged from 0.2 for Mental Health to 23.95 for General Health), except for Role Emotional (incremental point difference of 2.61). In the UK compared to age- and gender matched norms, study participants scored lower on all SF-36 scales. Specifically, point differences between UK male norms and UK male study population indicated a decrement ranging from 1.4 for the Mental Health domain to 61.25 for Role Performance domain. Similar results were reflected in the female group in whom, compared to UK norms, point differences were lower and ranged from 5.03 for Mental Health domain to 56.73 points in Role Performance. CHQ data revealed similar results. In the US, compared to age-matched norms, study participants scored lower on all CHQ scales (decremental differences ranged from 2.08 for Physical Functioning to 26.64 for Parent-impact Emotional) except Family Cohesion, General Behaviour, and Self-Esteem. In the UK, compared to age- and gender matched norms, study participants scored lower on all CHQ scales (reduced parent-impact Emotional score from 1.65 for Self Esteem to 33.03 for Parent-Impact Emotional) except Family Cohesion. Differences of these magnitudes are generally considered clinically significant. Further, UK study participants produced a utility score of 0.61. Summary/Conclusions. Results indicated that participating patients, SCD, or MDS currently undergoing infusion ICT showed much lower HRQoL scores compared to population norms, and particularly for General Health, Role Physical, and Parent-Impact Emotional. Reducing the burden of treatment on the patient by having an effective, well tolerated, and more convenient therapy would contribute to improving the quality of life of these patients.

**0810**

**ESTIMATED TOTAL ANNUAL COSTS OF INFUSED IRON CHELATION THERAPY IN THE UNITED KINGDOM**

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**Backgrounds.** Patients suffering from β-thalassemia, sickle cell disease, and myelodysplastic syndrome undergoing chronic blood transfusions are at risk for iron overload which, if not corrected by iron chelation therapy (ICT), can create serious organ damage and reduce life expectancy. Deferoxax (DFO) is the standard of care for the depletion of excess bodily iron. It has to be infused for 8-10 hours, 5-7 times a week. Although the clinical need for ICT is clearly established, less is known about the economic burden of DFO treatment. Aim. To estimate the total cost of ICT in patients from the perspective of the National Health Service. Methods. We enrolled patients with severe hemophilia A, age ≥ 18 years, at least 18 bleeds in the previous year, switching from on-demand treatment with a plasma-derived FVIII to prophylaxis, with 25 IU/Kg 3 times a week. Results. The mean cost to treat one bleed was 3,955 (median: 2,391). The incremental cost-effectiveness ratio, i.e. the cost for avoided bleed, was 4,675. At the end of the follow-up period, SF-36 showed a statistically significant improvement in patients quality of life in all domains (p<0.05). Concerning the Physical Component Summary score (PCS) and the Mental Component Summary score (MCS), patients on PP showed better results than those on ODP, although no significant difference was found for MCS. Results obtained with EuroQol-5D were comparable to those on SF-36, with significantly different Visual Analogue Scale scores after ODP vs. after PP (65.06 and 72.65 respectively, p<0.01). Summary/Conclusions. These findings showed prophylaxis with RefactoTM’s dose of 25 IU/Kg tiw in adults with haemophilia was effective and safe. Our cost-effectiveness results can represent the point of reference for other similar evaluations. Furthermore prophylaxis has provided a significant improvement in HRQoL and it should therefore be considered in a cost utility evaluation.

**0812**

**COST UTILITY ANALYSIS OF DEFERASIROX VERSUS DEFEROXAMINE FOR PATIENTS REQUIRING IRON CHELATION THERAPY IN THE UNITED KINGDOM**

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**Backgrounds.** Patients suffering from β-thalassemia (β-thal), sickle cell disease (SCD), and myelodysplastic syndrome (MDS) require lifelong blood transfusions and are at risk of iron overload. If they are not treated with iron chelation therapy (ICT), they can suffer serious organ damage and reduced life expectancy. Desferal, infused subcutaneously for 8 to 10 hours per day, 5 to 7 times per week, is the standard of care for the depletion of excess bodily iron. Exjade is a new once daily oral iron
chelator, which has recently been approved by the FDA and Swissmedic for the treatment of transfusional iron overload. Aim: To estimate the incremental cost per quality adjusted life year (QALY) of using Exjade instead of Desferal in patients with β-thal, SCD, or MDS who require iron chelation, from a UK NHS perspective.

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<tr>
<td>Incremental cost per QALY</td>
<td>£ 779</td>
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</table>

Methods: An aggregate annual cost of ICT with Desferal was informed by a primary study of 29 patients (11 β-thal; 14 SCD, 4 MDS; 31% male; mean age 50.6 ± 20.1 years, mean weight 54kg) from four UK treatment centers on the basis of chart reviews and interviews. Major resource items included drug costs, home delivery pumps and balloon infusors, and items relating to the use of portacaths. For Desferal, weighted mean prescribed annual dose frequency was 236, with a compliance rate of 83.7%; mean dosage was 37 mg/kg at a cost of £8.88/g. Exjade was assumed to have a prescribed frequency of 365 doses per year, with the same compliance rate as observed for Desferal (83.7%), and a dose of 20 mg/kg at a cost of £34/g. Unit costs (2004/2005 GBP) were applied. Costs related to monitoring were excluded, as were adverse events as a conservative assumption of equal compliance with Desferal and Exjade was defined. Annual utility values reflecting the impact of subcutaneous infusion compared with oral administration were estimated to be 0.61 and 0.85, respectively (Lawrence et al., ASH 2005). Results. In the base case analysis, Exjade has an extremely low incremental cost per QALY of £779, as shown in the Results. See Table. A range of one-way sensitivity analyses are also presented. Conclusions: The once a day orally administered Exjade appears to offer an extremely cost-effective alternative to the current infusion-based iron chelator Desferal.

11th Congress of the European Hematological Association

10814 A QUALITY MANAGEMENT SYSTEM FOR JACIE ACCREDITATION AT MINIMAL FINANCIAL COST

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Backgrounds: The Joint Accreditation Committee of the International Society for Cellular Therapy and the European Blood and Marrow Transplantation Group (JACIE) offers an accreditation programme to transplant centers that are willing to benchmark and optimize their transplant activities. As a backbone of the process, it is mandatory to change in day-to-day patient care, submission to this programme demands documentation of education and training, reporting of clinical outcomes and the formalisation of a Quality Management System (QMS). Commercial QMS solutions are readily available, but these lock-in and guarantees permanent open data availability with adjustable access control. The licence is intended to preserve the freedom to share the software with other users, the guiding of resolution and the delivering of reports to HOT (HOT-Open Tickets): a user-friendly open source trouble-ticket system. This allowed us to deliver, using the local intranet, a front end to an eventreporting database back end, and this with minimal development efforts and minimal cost.

Methods: The effectiveness evidence used for analysis was derived from a review of published clinical studies enrolling patients who failed fludarabine, except in the case of CHOP, where the limited data available were mostly based on patients with less advanced disease. Expected months in remission per patient treated was used to assess the health benefit of each therapy, and was estimated by multiplying the weighted overall response rate by the duration of response. In addition, for comparison between alemtuzumab IV and CHOP, life-months gained were calculated. Resource use associated with each intervention was based on the published literature, a previous patient level costing study conducted in the Netherlands in follicular lymphoma, and expert opinion. Unit costs for the Netherlands were derived from local hospital accounting systems, published tariffs, and listed wholesale prices. Sensitivity analysis was used to test the robustness of the findings. Unit costs were based on the 2003 price year. Results: Mean total cost of treatment with alemtuzumab IV was calculated to be approximately £23,281. Savings associated with a switch from IV to SC alemtuzumab are #3,085 with hematologist administration and higher with self-administration. Although cost of treatment with CHOP, #7,174, is lower than with alemtuzumab, in terms of health benefit the expected number of months in remission per patient treated is 3.61 months with alemtuzumab versus 1.59 months for CHOP. Cost of 12-dose rituximab, #80,155, is higher than alemtuzumab, and the expected time in remission is less at 1.98 months. Comparison of health benefits for each therapy with their cost shows that the mean costs per month in remission for alemtuzumab and CHOP are within a similar range: CHOP is #4,519 as base-case scenario, alemtuzumab SC 65,608, and alemtuzumab IV 66,449. For 12-dose rituximab the cost per month in remission is considerably higher at #15,195. When compared with a historical chemotherapy control, alemtuzumab is associated with a survival gain of approximately 8 months. Assuming this level of health benefit over CHOP would lead to an incremental cost per life-year gained for alemtuzumab IV of #24,160. Conclusions. This analysis shows that for each third-line CLL treatment that alemtuzumab IV is used instead of CHOP, additional cost to the payer would be on average #16,107, or less at #13,072 for SC administration by a hematologist. Benefit to the patient would be 8 months of survival gain, on average. The associated incremental cost per life-year gained for alemtuzumab IV over CHOP would be #24,160, well within the accepted range. Furthermore, when the costs and benefits of alemtuzumab are compared with high-dose rituximab monotherapy, alemtuzumab is both less expensive and more effective.
unreported. The software is free of charge, and installation takes around 15 hours of paid collaborator time. The solution can be shared on request, conform GPL. It is extendable for documenting educational JACIE needs. Screenshots will be presented. Results and conclusion A tool for event reporting was established at minimal cost through open source software. It guarantees indefinite availability of data and anticipates future legislation. It contributes to a quality management system as needed for JACIE accreditation.

Links
http://www.gnu.org
http://www.apache.org
http://www.php.net
http://hotopentickets.sourceforge.net
http://www.jacie.org

0815 ESSENTIAL THROMBOCYTHEMIA: ANAGRELIDE OR INTERFERON-ALPHA A COST-UTILITY ANALYSIS
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Backgrounds. Essential thrombocythaemia (ET) is a rare myeloproliferative disorder characterised by an abnormally high platelet count, leading to increased risk of thrombohaemorrhagic events. Platelet reduction therapy is indicated in high-risk patients, with hydroxyurea carbamide used as first-line treatment. However, up to 20% of patients are refractory or intolerant, and therefore require a second-line alternative. Busulfan, radiophosphorus (32P) and pipobroman are rarely used owing to their leukemogenic potential. Interferon-α (IFN-α) and anagrelide are two platelet-lowering agents thought not to possess this same risk. As resources for healthcare expenditure are finite, it is desirable to know which of these two options provides the best value. Aims. To assess the relative cost-effectiveness of IFN-α and anagrelide as second-line therapy for the management of ET in high-risk patients. Methods. A Markov model, capturing the risk of deep vein thrombosis (DVT), cardiac, stroke, and gastrointestinal (GI) bleed events, was developed to estimate the lifetime costs and quality-adjusted life-years (QALYs) gained from treating a notional 60-year-old female with anagrelide or IFN-α as second-line ET therapy. All data used to populate the model were extracted from the published literature. Results. Anagrelide as a second-line treatment for ET results in improved overall quality of life relative to IFN-α (12.35 versus 11.47 QALYs). The lifetime cost of anagrelide is higher than for IFN-α (£58,624 versus £45,702 [costs and benefits discounted at 3.5%]). Therefore, the extra cost per QALY gained from anagrelide compared with IFN-α is £14,805. This result is sensitive to assumptions on the impact of treatment side-effects and subcutaneous injections on quality of life. However, taking into account uncertainty within the input parameters, we estimate that for 89.5% of patients, the incremental cost-effectiveness ratio (ICER) will be below £30,000, the typical willingness-to-pay threshold for a QALY in the UK National Health Service. Conclusion. For high-risk patients suffering from ET, anagrelide is likely to be a more cost-effective second-line option compared with IFN-α.

0816 IMMUNOLOGIC RECOVERY AND GRAFT VERSUS HOST DISEASE AFTER NONMYELOABLATIVE STEM CELL TRANSPLANTATIONS
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Background. Non myeloablative stem cell transplant (NSCT) is an important therapeutic option for those patients (pts) who are not eligible to conventional transplant. The curative potential of these transplants are directly related to the anti tumor effect of the graft and rapidity of donor T cells engraftment. Methods. We have thus investigated the cellular immunologic recovery in 30 pts (20-64 years old) given peripheral blood stem cells from Matched Related and Unrelated Donors, mostly receiving a conditioning regimen based on fludarabine, Antithymocyte globulin (ATG) and Cyclophosphamid (or cytarabine or melphalan). Post grafting immunosupression usually consisted of cyclosporin and mycophenolate mofetil. The mononuclear blood cell subsets were assessed by flow cytometry; analyses were performed on days 30, 60, 90, 180 and 360 after SCT. Incidence of graft versus host disease (GVHD) and opportunistic infections was correlated with immune recovery. Results. We observed an early recovery of CD8+ T cell (d5) and NK cells (d22) whereas CD4+ T cells remain below the normal value until d500. This delay in CD4+ T cell recovery was not correlated with the total T cell number in the graft in our series. GVHD rate was similar to classical SCT - maybe with a lower severity- but mostly delayed compared to classical SCT. Our data show that a low rate of CD4+ T cells does not protect from GVHD, but this delay in T cell recovery might explain the late occurrence of GVHD in NSCT and also a later CMV-specific immune reconstitution translated into an increased frequency of CMV-reactivations. However, this did not lead to increased CMV diseases. We also observed a higher rate of CMV infections when CD4+ T cells were below 100/mL. Invasive fungal infections were not correlated with CD4+ T cells recovery in our study and mostly observed in patients receiving steroids for GVHD. 80% of patients in complete response after NSCT had developed a GVHD. Donor lymphocytes infusion was mostly useful to salvage relapsing who failed to present a GVHD after SCT. Conclusions. Our small series confirms the good tolerance of these NSCT, a similar immune reconstitution pattern to those seen after myeloablative SCT, a late and low rate occurrence of GVHD using ATG in the conditioning regimen, and an increased rate of CMV infections correlated with a low count of CD4+ T cells.

0817 LOW-DOSE METHOTREXATE AS SALVAGE THERAPY FOR REFRACTORY GRAFT-VERSUS-HOST DISEASE AFTER REDUCED-INTENSITY CONDITIONING FOR ALLOGENIC STEM CELL TRANSPLANTATION
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1Institut Paoli-Calmettes, Marseille, France

Corticosteroid (CS)-resistant GVHD is associated with high morbidity and mortality, and therapeutic options are limited for those patients. Also, elderly patients undergoing RIC allo-SCT are more exposed to the side effects of long term CS administration. Low dose methotrexate (MTX) therapy is a well established modality for GVHD prophylaxis. However, little is known about the role of this drug in CS-resistant GVHD. This pilot single center study investigated the role of MTX in a curative setting after failure of CS treatment in patients undergoing RIC allo-SCT. 20 patients received IV infusions of low dose MTX (5 mg/m2/infusion) at weekly intervals, for at least 4 weeks. Reasons for MTX administration were: CS-refractory acute GVHD, CS-refractory chronic GVHD, chronic GVHD exacerbation after CS taper, or CS severe side effects. Responses to low dose MTX infusions were assessed one month after the last infusion in each involved organ. 12 patients were treated for severe acute GVHD, while 8 patients received MTX for extensive chronic GVHD. Median age of patients was 51 (range, 22-60). Median time of administration of MTX was day +89 (range, 32-300). Of note, none of the patients received any other concomitant therapy for refractory GVHD. 13 patients responded to MTX administration (65%) with 5 complete responses (25%). Among the 12 patients treated for acute GVHD, 7 responded (58%) of whom 5 CRs (42%). 3 patients did not respond and died from resistant GVHD. Interestingly, 5 patients from the group of grade 3-4 acute GVHD responded. Among the 8 patients treated for chronic GVHD, 6 were responders (75%). In addition, MTX allowed a significant reduction of CS daily dosage ranging...
from 25% to 90%, as assessed one month after the last administration of MTX. With a median follow-up of 287 days, no increase of CS therapy was necessary among these 6 MTX-responding patients. In all, toxicity of low dose MTX administration was low (transient and mild reversible cytopenia in 3 cases, 15%). Among the 20 patients, 14 are still alive (70%) with a median follow-up of 293 (range, 65-513) days. Overall, 2 patients died of progressive disease, while 4 patients died from refractory GVHD. We conclude that low dose MTX is a well-tolerated, inexpensive and likely steroid-sparing agent that is worthy of further investigation in prospective trials for treatment of refractory GVHD, but also as frontline therapy in combination with CS.

### 0818

**OBSERVATION BASED EARLY WARNING SCORES TO DETECT IMPENDING CRITICAL ILLNESS PREDICT IN-HOSPITAL AND OVERALL SURVIVAL IN PATIENTS UNDERGOING ALLOGENEIC STEM CELL TRANSPLANTATION**

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**Backgrounds.** Observation-based early warning scoring systems have been developed to improve the outcome of critically ill patients by triggering early critical care intervention. To date none of these scoring systems have been validated in cancer patients or stem-cell transplant recipients. The aim of this study was to validate three established scoring systems in adult recipients of Allogeneic stem cell transplantation (Allo-SCT) and to determine their usefulness at predicting survival. Methods. We retrospectively analysed the physiological observations during the initial admission of patients undergoing Allo-SCT. Three different early warning scoring systems termed MEWS, PARS and LEWS (Table 1) were assessed.

<table>
<thead>
<tr>
<th>Table 1. Leads based modified early warning score (LEWS).</th>
<th>Score</th>
<th>3</th>
<th>2</th>
<th>1</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart Rate (beats/min)</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Systolic blood pressure (mmHg)</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Respiratory rate (min-1)</td>
<td>&lt;8</td>
<td>8-11</td>
<td>12-20</td>
<td>21-25</td>
<td>26-30</td>
<td>&gt;30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxygen saturation (%)</td>
<td>&lt;85</td>
<td>85-89</td>
<td>90-94</td>
<td>&gt;95</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiratory support</td>
<td>BiPAP/CPAP/Hi/Flow/Oxygen Therapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine output in last 4 hours</td>
<td>&lt;80</td>
<td>80-120</td>
<td>121-200</td>
<td>201-790</td>
<td>&gt;800</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Level of consciousness</td>
<td>Confusion</td>
<td>Alert</td>
<td>Reacts to voice</td>
<td>Reacts to pain</td>
<td>Unresponsive</td>
<td></td>
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</table>

**Results.** Charts of 43 patients (AL n=21, HD/NHL n=10, MM n=4, CML n=7, SAA n=1) with a median age of 40 years (IQR 29-49) were analysed. 29 of 43 patients received grafts from matched sibling donors and 16 of 43 received radiation-based full intensity conditioning. Impairment of respiratory function was the commonest (40 patients, 93%) event, usually deteriorating during the second week post-graft. All scores revealed high accuracy in predicting in-hospital survival (AUC in ROC for MEWS 0.915, for PARS 0.983 and for LEWS 0.988 respectively, p < 0.001). For all three scores the cut-off level associated with a high risk of in-hospital mortality was 7. Of eight patients with a LEW score of ≥7, 7 died during their initial admission, whereas no patient with a lower score died (PPV 88%, NPV 100%, specificity 97%, sensitivity 100%). Acute clinical deterioration during the initial admission appeared to have an adverse effect on overall survival after discharge: in-hospital survivors who reached a LEW score ≥8 during their admission had a median survival of 665 days (median survival not reached in patients with LEWS < 5, p=0.018). Conclusions. This is the first study to validate early warning scoring systems in Allo-SCT and demonstrate that these systems are highly predictive of in-hospital survival and overall survival post-discharge. The most likely time to develop clinical deterioration is the second week after graft infusion.

### 0819

**A DOSE FINDING STUDY OF IV BUSULFAN IN COMBINATION WITH CYCLOPHOSPHAMIDE AS CONDITIONING REGIMEN PRIOR ALLOGENEIC STEM CELL TRANSPLANTATION IN CHILDREN WITH MALIGNANT AND NON-MALIGNANT HAEMATOLOGICAL DISEASES: OPTIMISATION OF BUSULFAN TREATMENT**

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1Hopital Saint Louis, PARIS, France; 2Institut Gustave Roussy, VILLEJUIF, France; 3Hopital Debreusse, Lyon, France; 4Hopital Hotel Dieu, NANTES, France; 5Hopital la Timone, MARSEILLE, France; 6Hopital Necker-Enfants Malades, PARIS, France; 7Hopital Robert Debré, PARIS, France; 8Hopital d’Enfants Brabois, VANDOEUVRE LES NANCY, France; 9Institut de Recherche Pierre Fabre, BOULOGNE BILLANCOURT, France

**Backgrounds.** In pediatric patients (pts) oral busulfan (Bu) is often included in conditioning regimens prior to allogeneic (allo)-haematopoietic stem cell transplantation (HSCST). Bu has a narrow therapeutic window and under- or overdosing may have a fatal outcome. Bu clearance (Cl) is high in children and higher doses are needed to obtain an area under the curve (AUC) equivalent to adults. To optimise Bu treatment we defined (Nguyen L. et al BMT 2004) and assessed prospectively a new IV Bu fixed dosing allowing to 91% of 59 pts targeting AUC (900-1500 µM.min) without therapeutic drug monitoring (TDM) (Pharmacokinetic results were reported separately by G Vassal et al). We report here the clinical outcome of these children after allo-transplant. Aims. To investigate the safety of this new IV Bu dosing strategy, to assess whether these dosages are myeloablative and supportive for engraftment, and to evaluate the consequences of IV Bu dosage upon children clinical outcome Methods. Children (15 male/15 female) received IVBu-based Bu-cyclophosphamide (Cy, 200mg/kg) prior to HSCST. IVBu (over 2 h infusion) was given based on body weight: 1.0 mg/kg, 1.2 mg/kg, 1.1 mg/kg, 0.95 mg/kg, and 0.8 mg/kg for pts with <9 kg, 9 to <16 kg, 16-25 kg, >23-34 kg, and >34 kg strata of weight, respectively. Clonazepam was given as premedication. Indications for HSCST were: AML (n = 14, 12 CR1, 1 CR2, 1 PR), CML (n = 8), ALL (n = 1), MDS (n = 1); hemoglobinopatia (n = 6), and immunodeficiency (n = 5). Recipients aged from 0.3 to 17.2 years (median 7.2 y) received bone marrow containing 5.7×10⁹/kg CD34⁺ (range 1.1-28) from matched related (22/28) or unrelated (4/26) donors. Regimen-related toxicity (RRT) was graded according to NCI-CTC 2.0. Kaplan-Meier EFS and OS were evaluated. All had profound myeloablation, and all (28/28, 100%) engrafted at 21 days (range 12-47) for neutrophils > 0.5×10⁹/L, and 38 days (range 16-90) for platelets > 50.0×10⁹/L. Donor cells were documented in all recipients: 25/28 achieved complete chimerism (CC, >99% donor), and 3/28 were mixed chimeras but had mainly donor cells (>85%). No primary and/ or secondary graft rejection occurred. IVBu was well tolerated: no grade (G) IV toxicity; G III 14 pts (mainly stomatitis), and G I-II 24 pts. VOD incidence was 7% (95% CI: 0.9-23.5%) but none was severe. Grades I-II and III-IV a-GVHD rates were 46% and 4%, respectively. There was no death at day+100, and Cumulative TRM incidence at 2 years was 4%. Four pts died due to c-GVHD (n = 1), AML relapse (n = 3). After a median follow-up of 28.0 months (range 18.2-38.2) estimated EFS and OS median was 7% (95% CI: 0.9-23.5%) but none was severe. Grades I-II and III-IV a-GVHD rates were 46% and 4%, respectively. There was no death at day+100, and Cumulative TRM incidence at 2 years was 4%. Four pts died due to c-GVHD (n = 1), AML relapse (n = 3). After a median follow-up of 28.0 months (range 18.2-38.2) estimated EFS and OS rates were 85% + 14% for both probabilities. Summary/Conclusions. These results indicate that this new IV Bu dosing optimises allo-engraftment with CC in 90% of pts, is well tolerated resulted in a very weak TRM, which translated into promising survival.
DALCIZUMAB HAS POOR EFFICACY IN STEROID-REFRACTORY ACUTE GRAFT VERSUS HOST DISEASE: A SINGLE CENTRE EXPERIENCE WITH 12 ALLOGRAFT PATIENTS

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Backgrounds. Daclizumab, a humanised monoclonal antibody against interleukin-2 receptor, has been used in steroid-refractory acute graft-versus-host disease (aGVHD). Reported results were conflicting. Aims and Methods. We performed a retrospective audit of the outcome data of 12 consecutive allograft patients who had been treated with Daclizumab for steroid-refractory (to > 2mg/kg/d of iv Methylprednisolone) grade III-IV aGVHD from year 2000-2004 in our unit. All patients received standard anti-microbial prophylaxis, and Cyclosporin and Methotrexate GVHD prophylaxis, except for three reduced-intensity allografts who received cyclosporine alone. Clinical grading of aGVHD was performed according to standard criteria. 1mg/kg of iv Daclizumab was given on days 1, 4, 8, 15 and 22 and definition of treatment response as previously described (Przezportka 2000). Results. Twelve patients developed grade III-IV aGVHD after HLA-matched blood stem-cell allogeneic transplants, who consisted of 9 sibling (7 ablative, 2 reduced-intensity) and 3 unrelated (1 ablative, 2 reduced-intensity) allo grafts. Daclizumab was commenced after failure of iv Methylprednisolone at a median of 81/2 days (range: 3-28). These patients also received numerous (range: 3-7) concomitant GVHD therapies, including Steroids, Cyclosporin/Tacrolimus, Mycophenolate, and Etanercept (for gut aGVHD). Anti-thymocyte globulin (ATG) was additionally given for poor responders to Daclizumab in 6/12 patients. The only complete responder to Daclizumab (patient 7) died of subsequent exacerbation of gut GVHD, haemorrhagic cystitis and CMV viraemia. The single partial responder to Daclizumab (patient 7) died of subsequent exacerbation of gut GVHD. Anti-thymocyte globulin (ATG) was additionally given for poor responders to Daclizumab in 6/12 patients. The only complete responder to Daclizumab (patient 7) died of subsequent exacerbation of gut GVHD, haemorrhagic cystitis and CMV viraemia. The single partial responder to Daclizumab (patient 7) died of subsequent exacerbation of gut GVHD, haemorrhagic cystitis and CMV viraemia. The single partial responder to Daclizumab (patient 7) died of subsequent exacerbation of gut GVHD, haemorrhagic cystitis and CMV viraemia. The single partial responder to Daclizumab (patient 7) died of subsequent exacerbation of gut GVHD, haemorrhagic cystitis and CMV viraemia.

Conclusions. In contrast to initial published reports, allograft patients with severe steroid-refractory aGVHD had poor response and dismal outcome when treated with Daclizumab in our institution. It was our major concern that the poor survival may be contributed by the delay of more appropriate GVHD therapy and the aggravation of infectious complications. As a result, we have moved away from Daclizumab back to ATG since 2005. Novel GVHD therapies such as photopheresis, mesenchymal stem cells should be explored.

Table 1. Pre- and post-Daclizumab responses and outcome.

<table>
<thead>
<tr>
<th>Score</th>
<th>Skin GVHD Day 43 Pre-</th>
<th>Gut GVHD Day 43 Pre-</th>
<th>Liver GVHD Day 43 Pre-</th>
<th>Outcome</th>
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<tbody>
<tr>
<td>1</td>
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<td>12</td>
<td>2</td>
<td>1</td>
<td>3</td>
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Without any parenteral nutrition were 68% (45) patients, and 32% (20) patients have parenteral nutrition in median 11 days (3-22 days). Recovery of neutrophils(>1.0x10^9/L) was in median time 18 days, trombocytes (>20x10^9/L) in median 18 days. Complete chimerism (CC) was reached in median time 73 days in 49 (78%) patients, 2 (5%) patient still didn’t reach CC, 2 (5%) patients didn’t reach CC for short time after transplantation, the others didn’t reach CC because of rejection of graft (1), giving his autologous back up of stem cells for severe GVHD (1), relaps of disease (4pts=7%), death from other reason (5 infections, 1 bleeding). Twenty-two (35%) patients developed an acute GVHD: 9 patients maximal grade I, 8 patients maximal grade II, 3 patients maximal grade III, 2 patients grade IV. A chronic GVHD was presented in 28 (44%) patients (23 limited,5 extensive). Secondary rejection of graft occurred in 2 patients with unrelated donor. Fourteen patients had pre-emptive therapy of CMV. Any infection since day +100 had 28 (44%) pts, after day +100 51 (49%) patients. Twenty patients (51%) died, the causes of death: 10 (15%) relapses of disease, 3 (5%) infections, 4 (6%) GVHD,1(2%) toxicity, 2 (3%) from other reason. Early transplant related mortality (TRM) was 10% (6 patients:2 relaps, 1 GVHD, 1 bleeding, 2 infections), late TRM 5% (8 patients GVHD). Median time of overall survival for all patients (Kaplan-Meier) wasn’t reached, AML patients had 46 months, CLL patients 6.4 months and CML patients and B-NHL patients wasn’t reached it. Conclusions. RIC is associated with favorable outcome and low toxicity in patients in remission at the time of transplantation.

ALLOGENIC STEM CELL TRANSPLANTATIONS AFTER REDUCED INTENSITY CONDITIONING REGIMEN FLUDARABIN, BUSULFAN AND ATG (FRESENIUS)

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Introduction: Allogeneic stem cell transplantation with reduced intensity conditioning (RIC) is effective therapy for hematological diseases. Methods. This is a retrospective report about 68 patients (21 women, 42 men, median age 51 years (15-65) who underwent hematopoietic stem cell transplantation (HSCT) after reduced intensity conditioning regi-

lowest dose methotrexate as salvage therapy for refractory graft-versus-host disease after reduced-intensity conditioning allogeneic stem cell transplantation

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Corticosteroid (CS)-resistant GVHD is associated with high morbidity and mortality, and therapeutic options are limited for those patients. Also, elderly patients undergoing RIC allo-SCT are more exposed to the

Figure. Overall survival of patients AML, CML, NHL, CLL.
side effects of long term CS administration. Low dose methotrexate (MTX) therapy is a well established modality for GVHD prophylaxis. However, little is known about the role of this drug in CS-resistant GVHD. This pilot single center study investigated the role of MTX in a curative setting after failure of CS treatment in patients undergoing RIC allo-SCT. 20 patients received IV infusions of low dose MTX (5 mg/m²/day) at intervals of at least 4 weeks. The majorities of patients were conditioned with CsA/MTX. 61 patients (15.3%), all patients were categorized as high risk with t(9;22) and for SAA (n=13) cyclophosphamide. 6 complete responses. Among the 12 patients treated for acute GVHD, 7 responded (58%) of whom 5 CRs(42%). 3 patients did not respond and died from refractory GVHD. We conclude that low dose MTX therapy is a well-tolerated, inexpensive and likely steroid-sparing agent that is worthy of further investigation in prospective trials for treatment of refractory GVHD, but also as frontline therapy in combination with Cs.

**0823**

**β THALASSEMA MAJOR: BONE MARROW VERSUS PERIPHERAL BLOOD STEM CELL TRANSPLANTATION**

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**Backgrounds.** Most thalassemia births are now seen in developing countries where the socioeconomic status generally remains poor. Because of low birth weight and low birth weight, donor willingness is a prime profile. PBSC remains an attractive option for multiply transfused Thalassemia patients in terms of rejection, GvHD and disease free survival. **Methods.** 56 patients were transplanted from September 2000 - July 2005. 29 underwent BMT and 27 received PBSCT. **Results.** The 5 year OS was 58%; 65% and 43% in patients with family donors and MUD respectively. CML: The 5 year OS was 66%; for those with family donor 78% and MUD 46%. AML: The 5 year OS was 56%; for the patients with family donor 61% and MUD 46%. ALL: The 5 year OS was 40%; for patients with family donor 48% and MUD 37%. SAA: The 5y OS was 85%. GVHD: Acute GVHD occurred in 46.7%; aGVHD grade II-IV was 53.2%. The incidence of chronic GVHD was 34.4%; 21.9% limited and 12.6% extensive. Infections: Proven or probable invasive fungal infections were registered in 44 patients (12.2%). CMV infection and disease in 74 (18.6%) and 21 (5.5%) patients respectively. Mortality: Mortality before day 100 was 11.4% and 25% in the family donor and MUD groups, respectively. Overall non-relapse mortality was 29%. Conclusions: Our results compare favorably with previously published series, both single centre studies and multicenter studies.

**0825**

**EXTRACORPOREAL PHOTOPHERESIS FOR ACUTE AND CHRONIC GRAFT VERSUS HOST DISEASE**


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Graft vs host disease (GVHD) is the main immunological complication of hematopoietic transplants. Unfortunately pharmacological therapies do not always effectively control severe and progressive cases. Extracorporeal photopheresis has been proposed as an effective procedure for GVHD treatment. As an alternative to this treatment, we evaluated the action Extracorporeal photopheresis (ex vivo leukocytes irradiation with minimal doses of γ irradiation). Clinical, immunological and skin histological evolution of patients with acute and chronic GVHD who received only pharmacological therapy were compared with...
patients who received the pharmacological therapy plus Extracorporeal radiation therapy for 2 weeks. Apoptosis induction, phenotype of dendritic cell subsets and cytokine production were evaluated in vitro. Results. 15 patients with aGVHD and 15 with cGVHD were studied. In aGVHD grade IV neither treatment affected the long-term survival. Nevertheless, in 3/4 patients (75%) who received radiopheresis, diminution of clinical symptoms (gastrointestinal bleeding and skin rash) was observed since the first week of treatment, improving the quality of life. This effect was also observed at later in patients receiving only pharmacological therapy. In cGVHD, 7 patients received radiopheresis, 5 (71%), improved the skin pain since the first week and the skin sclerosis after 6-12 months. In one patient, control of cGVHD progression was obtained. None of these patients had previously received pharmacological therapy without control of the GVHD. 1/8 patients that received only pharmacological therapy, three improved (37.5%), in three (37.5%), were control of cGVHD progression and two got worse (25%). Histological skin follow-up showed that in aGVHD severity score were one grade lower on all radiopheresis cases evaluated. In patients that received pharmacological therapy only, 80% were the same grade, 20% got worse. For cGVHD skin biopsies were made after 6-12 months. Lower or same histological grade were observed in six patients who received radiopheresis and four patients with only pharmacological therapy, 87.5% and 30% respectively. Induction of apoptosis in cells that received radiopheresis was evaluated with DIOC-6/BE. No changes in the phenotype of dendritic cells differentiated from monocytes with IL-4+GM-CSF were observed. Summary. Better clinical and histological therapeutic effects were observed on patients who received Extracorporeal Radiopheresis. Multicentric studies will contribute to evaluate this therapy in a larger number of patients.

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0826
IMMUNOHISTOCHEMICAL EVALUATION OF INFILTRATING CELLS (CD4+, CD8+, AND CD56+) AND LANGERHANS CELLS IN SKIN OF GRAFT VERSUS HOST DISEASE PATIENTS

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Graft vs. host disease (GVHD) occurs in about 60-80% of allogeneic hematopoietic stem cell transplant recipients due to mismatching of major and minor histocompatibility antigens. Cutaneous involvement is the most frequent clinical manifestation of GVHD. In this regard, Langerhans cells (LC) have been shown to play an important role in the pathogenesis of GVHD as antigen-presenting cells through both the direct and indirect allo-recognition pathways. Thus, we carried out an immunohistochemical study to determine the characteristics of the infiltrating T cells (CD4+ and CD8+) and NK cells (CD56+) as well as LC (CD1a+) on skin biopsies of GVHD patients and their correlation with global severity scores. Forty-two patients were allo-transplanted between June, 1998 and December, 2002. Twenty-nine (69%) patients developed GVHD; among these, 15 (36%) developed acute GVHD, 5 (11.9%) developed chronic GVHD, and 9 (21.4%) developed acute and chronic GVHD. Immunohistochemical enumeration of CD1a+, CD4+, CD8+, and CD56+ cells were performed in paraffin-embedded punch skin biopsies taken mainly from the thorax. Among the 24 cases with acute GVHD, skin involvement was observed in 23/24 (95.8%) patients, most of them with G-I-II scores. Intestinal GVHD was observed in 20/24 (83.3%) patients with 15 (75%) patients with G-I-II scores and 5 patients (25%) with G-III-IV scores. Hepatic GVHD was observed in 8 (33.3%) patients, 5 (62.5%) of those patients with G-I-II scores. The number of LCs/mm2 in dermis and epidermis was significantly lower in cases with major global severity scores: Normal skin donors: (mean±SD) 15.6±1.6, acute GVHD G-I-II: 7.5 ± 8.8 and G-III-IV: 3.6±2.7 (p<0.05). An increase in the ratio of infiltrating perivascular was evaluated. CD8+ T cells were observed and it was inversely proportional to the number of LCs on epidermal and dermal layers of the skin. There was no increase of CD56+ NK cells in patients with acute GVHD as compared to normal controls. Figure 1. Extensive chronic GVHD was seen in 7/14 (50%) patients. The number of LC was similar in limited and extensive chronic GVHD (9.5±4.2 and 9±12.7, respectively). In de novo chronic GVHD, the number of LC was higher (15.2±4.6), than in progressive (7.3±4.0) or quiescent (2.7 ± 3.9) GVHD (p=0.05). Scleroderma-like presentation showed higher number of LC (9.7±1.8) as compared to lichen-like lesions (7.3±0).

No increase in the ratios of infiltrating epidermal and perivascular CD8+, CD4+ or CD56+ cells was observed. In summary, in acute severe systemic GVHD, a significantly lower number of LCs and higher number of CD8+ T cells were observed. These changes were not observed in chronic GVHD. This study indicates that skin CD8+ T cell/LCs ratios could be used as an additional tool for diagnosis and follow-up of GVHD.

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0827
DYNAMICS OF LINEAGE-SPECIFIC CHIMERISM IN PATIENTS AFTER NON-MYELOABLATIVE HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Backgrounds. Monitoring of engraftment by assessing chimerism has become routinely used method after allogeneic hematopoietic stem cell transplantation (HSCT). Information about the proportional degree of donor and recipient hematopoiesis and its dynamics in time is particularly important in patients receiving non-myoeloblate preparation regimens. Aims. The purpose of this study was to describe some aspects of correlation between persistence of lineage-restricted mixed chimerism (MC), complete donor chimerism (CDC), minimal residual disease (MRD), risk of graft versus host disease (GVHD) and relapse. We retrospectively analyzed data of chimerism obtained from 21 patients who underwent non-myoeloblate HSCT for chronic myeloid leukemia (CML) in our centre between June 1998 and December 2005 (827 chimerism quantification of whole blood samples, 259 lineage-restricted ones). The conditioning regimen consisted of fludarabine, busulfan and ATG Fresenius. GVHD prophylaxis was cyclosporine A with or without MMF. Methods. Assessment of chimerism was carried out by singleplex amplification of variable number of tandem repeat (VNTR) or short tandem repeat (STR) regions - mainly with capillary electrophoresis with fluorescence detection (apart from the beginning when densitometry was involved). Since September 2005 real-time quantitative polymerase chain reaction (RT-PCR) of insertion/deletion polymorphism was adopted. Detection of MRD was performed by competitive nested PCR and by reverse-transkriptase RQ-PCR. Individual leucocyte subsets (B cells, T cells, NK cells, granulocytes and monocytes) were fluorescence-activated cell sorter (FACS)-sorted according to corresponding antigens. Results. Observed patterns are mentioned bellow: 1) MC in whole blood along with MRD negative samples always means that autologous population comes from a lymphoid line. 2) MRD positive samples correlate with appropriate MC in granulocytes. 3) MRD positive samples along with CDC in whole blood are caused by limited sensitivity of VNTR/STR-PCR (repeatedly retrospectively verified by RQ-PCR, which showed microchimerism). 4) Achievement of CDC is preceded with CDC in T-cells. However CDC in T-cells is not necessarily followed by CDC in whole blood (failure of graft versus leukemia (GvL) effect). 5) MC (even in T-cells) does not always means protection from GVHD. Conclusions. Our preliminary data demonstrate that lineage-restricted chimerism analysis allows better understanding of hematopoiesis recovery after HSCT and can significantly contribute to interpretations of relations between chimerism and MRD or GVHD. On the other hand, however, the predictable value of chimerism and especially microchimerism must be further investigated.
ALLOGENEIC BONE MARROW TRANSPLANTATION IN PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

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Background and Aims. Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired chronic clonal hematological disorder of pluripotent stem cells. That disease may be complicated by myelodysplastic syndrome and leukemia conversion. Currently, allogeneic bone marrow transplantation (BMT) is the only curative approach. Patients and Methods. Between November 1990 and October 1998, six patients (2 F/4 M) with a median age of 26.5 years (range 17-38) underwent BMT for PNH at our institution. BMT was done due to severe progressive cytophenias in five patients and frequent recurrent hemolytic crisis in one. Median time from diagnosis to BMT was 40 months (range 7-58). Five patients were transfusion-dependent and three of them had received several lines of therapy before BMT (steroids, immunosupressors and danazol). Four donors were genotypic HLA-identical siblings, one was a non identical sibling donor (major mismatch to one class A HLA antigen) and the remaining one was an unrelated HLA-identical donor. Conditioning regimen consisted of busulphan (16 mg/kg) and cyclophosphamide (120 mg/kg). On day 0 all collected PBSCs (PBSC) from the same donor were infused without previous conditioning. Currently, five months after this PBSC infusion the patient is well with complete chimeria but with thrombocytopenia. Overall, four patients developed acute GVHD (one grade I and 3 grade III-IV). Chronic GVHD was extensive in four patients. Four patients are alive at +102, +118, +142 and +182 months, and two patients died at 11 and 97 months after BMT because of septic shock. Conclusions. Allogenic BM1 is a curative and suitable approach for selected patients with severe PNH. BUCY2 as conditioning regimen was able to eradicate the abnormal PNH clone. GVHD is the complication most frequently observed.

ALLOGENEIC STEM CELL TRANSPLANTATION IN MULTIPLE MYELOMA

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Backgrounds. HD chemo/radiotherapy followed by autologous stem cell transplantation (SCT) has been associated with improved outcome in MM. Unfortunately, following autologous SCT almost all pts had progressive disease. Aims. We evaluated the outcome of 29 pts (17 M, 12 F) with stage III MM treated with hematopoietic SCT. Twenty-three, 6 and 1 pts underwent a matched sibling donor allogeneic transplant after a reduced intensity conditioning regimens (RIC), a matched sibling donor SCT and an unrelated hematopoietic SCT, respectively. Twenty-two pts were treated with autografting followed by reduced intensity conditioning allotransplantation. All these patients received HD Melphalan (200 mg/m²) followed by autologous PB-SCT. After a median of 90 days, the pts underwent RICT (Fludarabine + 2 Gy TBI). Acute GVHD prophylaxis consisted of MM and cyclosporine. Chimerism analysis was performed using STR-PCR and donor engraftment was evaluated at day +15,+30,+45,+60,+90 on unfractonated BM cells. All pts received a HLA identical donor mobilized PBSCs and the graft contained a median of 3,8×10^6(range 1-6,8)× cells/kg body weight. After RICT, on day +15, 3 (13%) pts showed a complete donor chimerism; on day +90, 21 (90%) showed a complete donor chimerism; two pts with mixed chimerism received a DLI on day +80 and one of these achieved full donor chimerism. Results. Grade II-III acute GVHD occurred in 4 pts (17%) but no patient died. Five patients (22%) developed a mild and 6 (26%) an extensive chronic GVHD. After RICT 8 pts (35%) achieved CR and they are in CCR at +57,+57,+51,+49,+46,+20,+14 and +15 months; 2 (9%) pts show near CR and 3 (13%) are in PR. Ten pts not in CR showed a progressive disease and six of these died. With a median follow-up of 22 months, 17 (74%) are alive. Six and one patients received a related and unrelated hematopoietic SCT, respectively. The pre-transplant high-dose preparative regimen included CY+TBI. On day 0 all collected PBSCs were infused. GVHD prophylaxis included cyclosporine and short-course methotrexate; ATG was added in the unrelated transplant. All patients showed a complete donor chimerism at the time of engraftment. Grade II-IV acute GVHD occurred in 2 pts and 1 of these died. All pts developed mild chronic GVHD. One patient relapsed and died 24 months after allogeneic SCT. To date, 5 patients are alive and 3 of them are in CCR at +28 +4 (ALLO) and +21 (MUD) months. Conclusions. We demonstrated that survival after allogeneic transplantation is favourable: 74% of all pts achieved CR or PR. The 100-day TRM was low (only 4%) and no patient died after RICT. Pancycopenia after RICT was minimal and sustained allogeneic stem cell engraftment occurred in 95% of patients. A good correlation between GVHD, full chimerism and remission was found. All patients in CR or NCR developed acute/chronic GVHD and the presence of GVHD correlated with a lower relapse rate. In older patients (RICT, ALLO TMO and MUD) the achievement of CR was gradual and a constant regression of the monoclonal band was observed.

INDUCTION INSTEAD OF INDUCTION: NON-INTENSIVE AML/MDS TREATMENT WITH LOW-DOSE DECITABINE PRIOR TO REDUCED-INTENSITY CONDITIONING AND ALLOGENEIC BLOOD STEM CELL TRANSPLANTATION OF OLDER PATIENTS

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Patients (pts) undergoing allogeneic stem cell transplantation for AML or high-risk MDS usually receive standard induction chemotherapy first, with the goal of achieving a complete remission (CR) or at least best response to conventional chemotherapy. This disease control is often necessary to bridge the time needed for identifying an unrelated donor (n=7). One pt died before engraftment on day +13 due to aspergillosis prohibiting or compromizing subsequent allografting. For the remaining pts, non-intensive treatment with low-dose azanucleosides has recently been evaluated as an alternative for successful allografting following RIC (Bertz et al., J Clin Oncol 21:1480, 2003). However, prior induction chemotherapy may result in toxicities and prolonged cytophenia, related severe infection like aspergillosis prohibiting or compromising subsequent allografting. Recently, non-intensive treatment with low-dose azanucleosides has been developed for older pts with high-risk MDS. Low-dose Decitabine (DAC) shows a particularly interesting activity in inducing cytogenetic remissions in MDS pts with poor-risk cytogenetics (38%, Lübbert et al., Br J Haematol 114:349, 2001). The role of DAC prior to allografting has not yet been determined. Here we report our single-center experience of 9 consecutive pts treated by low-dose Decitabine (DAC) in MDS (n=6) or AML (n=3). The median number of DAC courses given was 5 (range 1-13) with CR and PR as best response in 4 and 1 pt respectively, stable or persistent disease in 3 and 1 pt, resp. Allografting was either done as consolidation at time of best response (n=4) or as salvage for relapsed/refractory AML/MDS (n=5). Median age at time of allografting was 65 years (range 63-71). After conditioning with either TBI (n=7) or Flu/2 Gy (n=2), pts were transplanted from either matched sibling (n=2) or unrelated donor (n=7). One pt died before engraftment on day +13 due to infection. The other 8 pts engrafted, and no unexpected toxicities were noted. 5/6 pts have died either due to relapse (n=3) or infection in CR (n=2). Three pts are alive in CR at the time of this report (+3,+5,+17 months following transplant). Prior to transplantation two of them were in PR and CR following 2 and 8 courses of DAC, respectively, one had beginning relapse of AML after 13 courses. In conclusion, we show that RIC and allografting in older pts with AML/MDS following DAC treatment is feasible, and no unexpected toxicities have occurred. DAC pre-treatment seems to induce improvement in karyotype and remission of myeloid neoplasia with negligible nonhematopoietic toxicity, thus bridging the time period of donor search prior to allografting. Since even multiple courses of low-dose DAC are unlikely to be curative without subsequent allografting, the allogeneic option in these pts should be explored more often. Response to DAC and transplant risk-assessment (comorbidities, performance status etc.) will likely be major determinants of optimal timing of allografting in this setting.

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**0831**

**TACROLIMUS AND METHOTREXATE FOR THE PROPHYLAXIS OF GRAFT-VERSUS-HOST DISEASE AFTER UNRELATED DONOR CORD BLOOD TRANSPLANTATION FOR ADULT PATIENTS WITH HEMATOLOGIC MALIGNANCIES**

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**Backgrounds.** Although allogeneic cord blood transplantation (CBT) has been increasingly used as a therapeutic option for hematologic malignancies, the prophylaxis of GVHD has varied significantly among different studies and has included cyclosporine A (CSA) alone or in combination with prednisolone/methylprednisolone, short-term methotrexate, or anti-thymocyte globulin. Aims. We present the outcome of CBT for adult patients who received tacrolimus and short-term methotrexate (MTX) for GVHD prophylaxis. Patients and Methods. Eighteen patients with hematologic malignancies underwent cord blood transplantation (CBT) from unrelated donors after having been conditioned with myeloablative (n=13) or reduced-intensity (n=5) regimens, and received tacrolimus and methotrexate (15 mg/m² on day 1, 10 mg/m² on days 3 and 6) as a graft-versus-host disease (GVHD) prophylaxis. The median number of nucleated cells of infused cord blood was 2.66×10⁷/kg of patient body weight. Results. Engraftment was achieved in 16 of the 18 patients. The median time to absolute neutrophil count ≥0.5×10⁹/L was 21.5 days (range 17-32), and the median time to platelet count ≥2.0×10⁹/L was 36 days (range 26-57). Of the 16 evaluable patients, 5 and 8 patients had grades I and II acute GVHD, respectively, and none had grades III/IV acute GVHD. The cumulative incidence of grade II acute GVHD was 44.4%. Chronic GVHD occurred in 7 of 15 evaluable patients (limited-type 3, extensive-type 4). Infectious complications were common, including sepsis in 10 patients, CMV disease in 3 patients, and fatal invasive aspergillosis in 1 patient. Of the 18 patients, 14 were alive and disease-free between 175 and 1514 days after CBT (median 746 days), and the probability of disease-free survival at 2 years was 79.1%. Conclusions. Our results suggest that tacrolimus and short-term methotrexate effectively prevent the occurrence of severe acute GVHD after unrelated CBT, and could contribute to a higher survival rate, although the management of infectious complication is essential.

**0832**

**INCREASING MIXED CHIMERISM DETECTED WITH SHORT TANDEM REPEATS DEFINES A GROUP OF PATIENTS WITH POOR OUTCOME AFTER REDUCED-INTENSITY CONDITIONING ALLOGENEIC PERIPHERAL BLOOD STEM-CELL TRANSPLANTATION WHICH CAN BE IMPROVED BY IMMUNOTHERAPY**


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**Introduction.** Recent studies indicate that patients with an increasing mixed chimerism after allo-PBSCT have a significantly enhanced risk of relapse. However, as long as we now, none of them have focused on RIC transplantation. Aim: To study the relationship between the degree of chimerism and the frequency of relapse, graft rejection, graft-versus-host-disease (GVHD), overall survival (OS) and event free survival (EFS) in patients who have received RIC allo-PBSCT. Patients. 102 consecutive stem cell transplant recipients (SCT) with peripheral blood stem cells from identical MHC sibling donors with reduced intensity conditioning at a single center were included in the study. Their characteristics were: median age 53 (23-69); Male/Female: 64/38; Sex disparity: 50%; Diagnosis: 21 MM, 19 NHL, 17 AML, 14 MDS, 11 CLL, 8 HL, 5 ALL, 4 CML, 2 CMID, 1 CML-LL. Two patients died prior to be evaluated, while the remaining cases were valuable for acute GVHD (cGVHD). In addition, 78 patients were included in the analysis for chronic GVHD (cGVHD). 77 were analysed by serial and quantitative chimerism analysis at days +28, +56 and +100. The mean follow-up was 385 days (21-2502). Methods. After genomic DNA extraction from bone marrow samples, PowerPlex®16 System kit (Promega Corporation, Madison, WI) was used to amplify 16 STR regions (15 plus gender marker, Amelogenin). The amplified products were analysed using GeneScan 2.1 (Applied Biosystems, Foster City, CA) after electrophoresis in the ABIPrism 377 (Applied Biosystems). For statistical analysis, the χ² and t-Student tests were used. Log-rank and Kaplan-Meier analyses were used to compare differences between survival curves. Multivariate analysis was carried out according to the cox-regression method. Criteria to define the chimerism status were the previously described by Bader et al (JCO, 2004, 22:1696): Complete chimerism (CC) - No autologous cells at any time after transplantation. Low-level mixed chimerism (LL-MC) - Weak (<5%) autologous signals. Decreasing mixed chimerism (de-MC) - Autologous signals decreasing >5% during follow-up. Increasing mixed chimerism (in-MC) - Autologous signals increasing >5% during follow-up. Results. 56/77 revealed CC or LL-MC; in-MC was found in 15 patients and de-MC in 6 patients. Relapse was significantly more frequent in patients with in-MC (12 of 15) than in patients with CC/LL-MC (14/56) or de-MC (2/6; p<0.001). The probability of 5-years EFS was 41% for all patients, with 7% for patients with in-QM and 51% for the rest patients (p<0.001). Within the 15 patients with in-MC, 6 received additional immunotherapy (DLI or bortezomb). This latter group had a significantly higher 5-year OS (67%) than those who did not receive immunotherapy (11%, p=0.05%). Regarding chronic GVHD, patients with CC/LL-MC/de-MC have more incidence of cGVHD (70%) than those with in-MC (15%, p=0.001). Conclusion: Serial analysis of chimerism reliably identifies patients at high risk to relapse. Accordingly, patients with increases MC should be actively treated because they are on high risk of relapse.

**0833**

**SERIAL ANALYSIS OF WHOLE BLOOD CELL AND CD3+ T-LYMPHOCYTE CHIMERISM FOLLOWING ALLOGENIC STEM CELL TRANSPLANTATION WITH ALEMTUZUMAB CONTAINING REDUCED-INTENSITY-CONDITIONING**


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Serial analysis of haemopoietic chimerism can be used to predict outcome after allogeneic SCT using a RIC regimen and guide post-transplant intervention. Although alemtuzumab is increasingly used as a component of RIC regimens, there have been few studies of its impact on chimerism status post-transplant. We have therefore measured chimerism following allogeneic SCT in whole blood/marrow nucleated cells (WB) and magnetically selected CD3+ T-lymphocytes in 64 patients with lymphoid (8 high-grade NHL, 18 low-grade NHL, 5 myeloma, 3 mantle cell lymphoma, 9 Hodgkin’s lymphoma) or myeloid malignancy (14 AML, 3 MDS, 1 myelofibrosis, 3 CML) conditioned with alemtuzumab and either fludarabine with melphalan (n=46), BEAM (carmustine, etoposide, cytarabine and melphalan) (n=16) or fludarabine/busulphan (n=2). Forty-seven patients received a transplant from an HLA compatible sibling donor and 17 from a matched unrelated donor. All patients achieved neutrophil and platelet engraftment. Donor chimerism was quantified within the first year post-allograft as well as following donor lymphocyte infusions (DLI) by FISH or PCR-based analysis of polymorphic microsatellite regions. 85% of patients demonstrated full donor chimerism (FDC, defined as ≥95% cells of donor origin) in WB within the first 90 days post-transplant. By contrast DLI was only present in the CD3+ compartment of 45% of patients. The proportion of patients with WB DLI declined to 64% by 12 months post transplant whilst the proportion of patients with CD3+ DLI remained constant. Thirteen patients received DLI using escalating CD3+ doses for management of mixed chimerism (MC) including 6 with evidence of disease relapse. Following DLI 7 patients achieved FDC in WB and CD3+ compartments and 4 failed to switch to DFC. Seven patients developed acute GVHD post-DLI. Acquisition of FDC in the CD3+ compartment within 90 days post-transplant correlated with the presence of acute GVHD (p=0.005). Sixteen patients relapsed of whom 13 exhibited MC in WB or CD3+ compartments. Three patients relapsed despite the presence of FDC in WB and CD3+ cells. There was a trend towards improved disease free survival in patients who achieved FDC in WB within 90 days of transplantation compared to patients with MC (median 30 months v 11 months respectively). These data define a different pattern of WB and CD3+ chimerism after alemtuzumab regimens compared with post-relept RIC regimens and confirm a correlation between chimerism status and outcome post-transplant.
With the engraftment of allogeneic transplantation, the patient becomes a real chimera, because of the cohabitation in the same person of a genetic patrimony coming from two different people: the patient (pt) and the donor. The periodic control of chimerism is very important for several reasons. It identifies the cellular population's type present after transplantation, it allows the right somministration of immunosuppressive therapy, supporting the reaching of engraftment, and the timely identification of a possible disease's relapse. We have investigated the kinetics of engraftment in 133 pts with different malignancies. Seventy nine (median age:47 range 22-62) received reduced conditioning regimens: 22 Flu/Mel, 26 Flu/Cy and 29 Flu/TBI and 54 pts (median age:33 range 10-58) received myeloablative conditioning regimens. We have also evaluated if CD34 cell dose influence engraftment. Due to its high sensitivity, chimerism's valuation is performed using multiplex PCR coamplification of 16 Short Tandem Repeat loci in a single reaction. Donor/recipient cell population ratio was detected by calculating peak area of PCR products for each informative marker. The median number of informative alleles was 6 (range 3-9). We have evaluated the number of patients that have reached the complete chimerism (CDCa≥95% donor's cell) at days +15, +30, +90, +180, +360 and so on. In the subgroups of pts that received non myeloablative conditioning regimens the outcome was respectively: 20/79 (25%), 30/79 (38%), 69/79 (87%) 74/79 (94%). We have only show that engraftment's kinetics of non myeloablative transplantation is more gradual in time compared to the myeloablative transplantation. In this last one, the engraftment is more rapid and the complete chimerism is already reached on the 30th day. In non myeloablative transplantation, donor engraftment was evaluated at day +15, +30, +90 and so on, in three subgroups of pts that have received different CD34 cell dose: <2×10^6/kg, 2×8×10^6/kg; and >8×10^6/kg. At the day +15 the kinetics of engraftment resulted significantly correlated to dose (p=0.028), while from the day +30 it didn’t significantly differ in the three subgroups (p>0.5). Conclusions: The valuation of the transplantation's kinetics of engraftment has shown that in non myeloablative transplantation is normal to have a mixed chimera with tolerance host versus transplantation and transplantation versus host, for this reason we suggest the importance of the periodic control of chimerism in order to modify the immunosuppressive therapy in favour of the engraftment and to identify immediately the disease’s relapse. The CD34 cell dose has a noticeable effect only in the early kinetics donor chimeraism (1 to 15 days).

Hematopoietic Recovery After Low Intensity Conditioning Transplants and Standard Allogeneic Hematopoietic Stem Cell Transplantation (HSCT)-COMPARATIVE ANALYSIS

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Aim. To compare hematopoietic reconstitution after low intensity conditioning transplants and standard allogeneic hematopoietic stem cell transplantation (HSCT). Methods. We retrospectively analyzed the kinetics of cytopenia of 50 consecutive patients treated with HSCT during a 60 day posttransplant period. Twenty four patients were treated with a low intensity regimen (Fludarabine, 2 Gy total body irradiation) and 26 patients with the standard conditioning regimen. Patients who received the low intensity HSCT were analyzed in two groups, patients with engraftment of donor hematopoiesis and those who rejected the graft. Results. Patients treated with low intensity conditioning, regardless of its outcome, experienced significantly less severe cytopenia than the patients from the control group. Except for reticulocytes, the development of cytopenia was significantly slower in these patients, and the duration of severe cytopenia was significantly shorter. However, full neutrophil recovery (absolute neutrophil count >1.0×10^9/L) took longer in patients with low intensity HSCT. Conclusions. The kinetics of cytopenia and hematopoietic recovery conditions are significantly different from standard HSCT. There is no difference in the initial hematopoietic recovery between patients with or without engraftment after low intensity conditioning. This indicates that the onset, severity, and duration of the cytopenia are influenced primarily by the intensity of the conditioning and by the immunosuppressive regimen after transplantation. Effects are more pronounced for neutrophils than for platelets and reticulocytes.
0837
CYTOMEGALOVIRUS INFECTION AFTER ALLOGENEIC STEM CELL TRANSPLANTATION WITH REduced-INtENSITY CONDITIONING REGIMEN
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Background and aim: Cytomegalovirus (CMV) infection remains one of the most important complications for patients (pts) undergoing allogeneic stem cell transplantation (allo-SCT). We evaluated incidence and outcome of CMV infection after allo-SCT with reduced-intensity conditioning (RIC) regimen. Methods. 30 consecutive pts (male: female = 17:13) aged from 38 to 67 years (median: 57) were allografted with bone marrow (5) or peripheral blood stem cells (27) from HLA-identical sibling donors from 2000 to 2005. The underlying hematologic malignancies were: acute myeloid leukemia (AML 8), myelodysplastic syndrome (MDS 7), non-Hodgkin lymphoma (NHL 6), multiple myeloma (MM 5), chronic lymphocytic leukemia (CLL 2), idiopathic myelofibrosis (MF 1) and chronic myeloid leukemia (CML 1). RIC regimens performed were: TT-EDX (17), FLU-TT-EDX (6), TBI 200 cGy (6), and FLU-TBI (1). CMV donor/recipient status was: positive/positive (27), positive/negative (2), negative/positive (1), negative/negative (0). All pts were weekly evaluated with CMV pp65 antigenemia assay for at least 1 month; thereafter antigenemia was determined only when a CMV infection was suspected due to clinical or biochemical features. The decision to switch from prophylactic to preemptive therapy was made on the basis of two consecutive positivity or first positivity > 5/200,000 cells. Acyclovir was given as CMV prophylaxis; preemptive therapy consisted of ganciclovir, valganciclovir, foscarnet or cidofovir. Results. A positive CMV antigenemia was detected in 10 pts; all of them were seropositive for CMV before allo-SCT. 4 pts had AML, 2 NHL, 1 CLL, 1 MM, 2 MDS. The incidence of CMV infection was 10/30 (33,3%). 7 pts presented only one episode of CMV reactivation, 1 patient two episodes and 2 pts three episodes. Median time of first CMV positive antigenemia was 52 days after allo-SCT (range: 22-356), particularly 8 pts had CMV reactivation before 100 days post SCT. Median positive cells at the first appearance of antigenemia was 3/200,000 cells (range 1-14). Overall, we reported 15 episodes of CMV reactivation, 9 before and 6 after 100 days post allo-SCT. Pts who developed late CMV reactivation showed contemporaneous chronic GVHD and disease relapse. Only 2/15 (13%) episodes were treated. Anti-CMV drugs employed had similar effectiveness with a median time of CMV clearance of 15 days. None developed CMV disease nor died for CMV infection. 5/10 pts who suffered CMV infection are still alive; while 5/10 died for disease progression (4) or GVHD (1). 13/20 (65%) pts without CMV infection are still alive. In the same years, 33 pts received a myeloablative allo-SCT from HLA identical donor: among them 6 pts developed a CMV infection for an incidence of 18%. Conclusions. In our group of pts transplanted with RIC allo-SCT, the incidence of CMV reactivation was 33,3%. Pre-emptive therapy was effective and no patient developed CMV disease.

0838
EARLY AND LATE COMPLICATIONS OF FAMILIAL ALLOGENEIC BONE MARROW TRANSPLANTATION. A SINGLE PAEDIATRIC CENTER EXPERIENCE
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Backgrounds. Improvement in survival rates after bone marrow transplantation (BMT) have sharpened the need for research on adverse events after treatment. These sequelae can have negative effects on quality of life of survivors and when severe are cause of morbidity and delayed mortality. The current study assesses the incidence of early and late effects by a single paediatric institution. Methods. Between January 1995 and December 2005, forty-one consecutive familial allogeneic BMT were performed in thirty-eight children (3 patients received a double transplant), with various haematological diseases (14 ALL, 11 AML, 1 CML in CR, 5 FEL, 2 MDS, 3 AAS, 2 NHL, 5 congenital haematological disorders). The median age of children was 9 years (8 months-15 years), 28 males and 10 female. The stem cell source was bone marrow (BM) in 33 cases, peripheral blood (PB) in 6 patients and cord blood (CB) in 2 children. Acute GVHD prophylaxis was performed by cyclosporine added to short-course MTX in immunocompetent patients. Conditioning regimen was diversified based on primary diagnosis. The mean early complications analysed were acute or chronic GVHD, veno-occlusive disease (VOD) and thrombotic microangiopathy (TTP), infections and haemorrhagic cystitis. All patients underwent a careful long-term follow-up for a rapid identification of endocrine dysfunctions, organ-specific sequelae or secondary malignancy. Results. The median dose of nucleated and CD34+ cell infused were 10,8 × 10^6/kg and 5,2 × 10^6/kg in PB-SCT and 4,6 × 10^6/kg and 3,4 × 10^6/kg in BM-SCT, respectively. Median time to neutrophil recovery more than 500/ml was 10 days for PB and 12 for BM while platelets engraftment more than 30,000/ml occurred in 18 days in PB and 21 days in BM (p=0.02); all patients showed a complete attachment. The total incidence of transplant related mortality (TRM) and disease relapse was 7,8% and 17%, respectively. The OS, DFS and EFS of our patients were 76%, 79% and 70%, respectively. The occurrence of grade III-IV acute GVHD and extensive chronic GVHD was of 17% and 7,8%, respectively, that not influenced significantly OS. VOD and TTP were observed in 14,6% and 4,8% of transplant, respectively. Severe infections, CMV reactivation and grade III-IV haemorrhagic cystitis were respectively 12%, 17% and 14,6%. Long-term complications were observed in 60,5% of patients, prevalently endocrine sequelae (47,5%). No data are available on fertility or sexual dysfunctions, for still young age of patients. In a median follow-up of 37 months (range 5-125 months) from transplant, no secondary malignancies were observed. Conclusions. These results suggest that familial allogeneic BMT is an effective therapeutic procedure in most paediatric haematological disease. Nevertheless, it is burdened by high incidence of late effects that often affect negatively the quality of life of survivors. Then, we believe that the patient and family have to be well informed before transplant about all possible complications of these treatment. Duty of clinicians is a careful, prolonged follow-up for a precocious identification and treatment of long-term effects. Future studies should focus on strategies aimed at reducing late sequelae, probably using conditioning regimen at reduced intensity.
Cytokines and growth factors

**0839**

**ERYTHROPOIETIN (EPO) AS AN IMMUNOMODULATORY AGENT: EPO-ASSOCIATED B CELL PROLIFERATION**

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Erythropoietin (Epo) is the key hormone regulating erythropoiesis. Recombinant human Epo (rHuEpo) is thus used as a major treatment for various types of anemias. Studies in the past decade have revealed extramedullary sites of Epo production, along with abundance of Epo receptors in various tissues and cell lines, suggesting that this hormone may actually have pleiotropic activities. Our previous studies have implicated Epo as an anti-neoplastic agent in murine multiple myeloma (MM) models (Mitelman et al., PNAS 2001; Katz et al., 2005). The implication that CD8 type T lymphocytes are involved in the anti-neoplastic effects of Epo raised the possibility that Epo has a wide-range of immunomodulatory effects. We thus investigated the effect of Epo on the immune system, focusing on two experimental models. (a) Epo-injected mice as compared to their diluent-injected counterparts (b) transgenic mice constitutively overexpressing Epo (termed tg6) as compared to their age-matched wild-type siblings. In both experimental models we found increased B-cell responses related to Epo effects. Namely, Epo-treated and Epo transgenic mice displayed higher proliferative responses to lipopolysaccharide (LPS) in vitro, indicating Epo-associated improved B cell functionality. On the other hand, in-vitro stimulation of splenocytes with T cell specific mitogens (e.g. Concanavalin A and anti-CD3) elicited less proliferation in Epo-treated and in Epo-overexpressing mice, as compared to their control non-treated and wild type counterparts, respectively. In accordance with these data, FACS analysis of splenocytes from the Epo transgenic mice demonstrated a moderate decrease in CD4-positive T cells and moderate increases in the CD19-positive B cells and in the natural killer cells. These data are supported by an improved Epo-associated response to antigen. Taken together, we propose that Epo acts as an immunomodulator, thus rendering it a potential adjunct agent in the treatment of various diseases.

**0840**

**IL-10 GENE POLYMORPHISM INFLUENCE THE CLINICAL COURSE OF NON-HODGKIN'S LYMPHOMA**

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**Backgrounds.** Non-Hodgkin's lymphomas (nHL) are heterogeneous group of lymphoproliferative disorders. In the North America and Europe the most frequent nHL are the B-cell lymphomas. Interleukin-10 (IL-10) is an important anti-inflammatory cytokine, mainly produced by Th2 and B lymphocytes. Many studies have shown that IL-10 may be associated in the pathogenesis of lymphoid disorders. Production of many cytokines is related to its gene promoter polymorphism and this polymorphism could be associated with aggressive form of nHL. We have recently demonstrated that the association between the presence of TGF-β1 high producer genotype - TGF-β1 +869 T/C (Leu10Pro) / 915 G/G (Arg25Arg) and TGF-β1 -869 T/T (Leu10Leu) and 915 G/G (Arg25Arg) - and the extra nodal manifestation of the non-Hodgkin lymphoma (Cytokine 2006). Aim: In this present study IL-10 gene polymorphisms were analysed in 55 NHL patients and 50 controls. Methods. IL-10 gene promoter polymorphisms at positions (-1082 A/G, -819 C/T, -592 A/C) were determined by PCR-SSP technique employing commercial primers (One Lambda, Inc. Canoga Park, CA, USA). Results. Only a slight prevalence of ACC among patients as compared to controls was observed (32/55 vs. 21/50, p=0.07). Interestingly, this genotype was more frequently detected in patients with more aggressive disease (17/25 vs. 15/52, p=0.04) and in those with 2 or more extra nodal sites of the disease (11/14 vs. 21/41, p=0.07). To assess if IL-10 ACC genotype (associated with lower IL-10 production) constitutes an independent risk factor of more aggressive course of nHL (multivariate logistic regression analysis was performed). IL-10 ACC genotype together with other clinical and biological factors (patient sex and age, stage and aggressiveness of the disease, presence of B symptoms, serum LDH level) was subject ed to this multivariate statistical analysis. Multivariate analysis confirmed the role of ACC genotype as a risk factor of more aggressive nHL manifestation (OR=3.57, p=0.05) in addition to the elevated LDH480 level (OR=3.95, p=0.04). Next analysis performed with respect to the number of extra nodal sites showed, although not highly significant (0.05< p<0.1), influence of the presence ofACC genotype. **Conclusion:** The low producer genotype of anti-inflammatory Th2 cytokine IL-10 was found to be associated with an unfavorable course of nHL.

**0841**

**EFFECT OF PTH (1-34) ON HOMING OF HEMATOPOIETIC STEM CELLS**

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**Background.** Osteoblasts are one of the key components of hematopoietic stem cells (HSC) niche within bone marrow. It was shown that parathyroid hormone (PTH) treatment leads to simultaneous increase in the trabecular osteoblast generation and in the HSC number. PTH is used in pharmacological concentrations for treatment of patients with osteoporosis. Influence of PTH on properties of hematopoietic precur sor cells of different stages of maturation is the point in question. Aims. The goal of this study was to test the ability of stromal microenvironment of PTH treated mice to maintain successful engraftment of HSC.

| Table 1. Homing of CFU-S and CAFC-28 in BM and spleen. |
|-------------|----------------|----------|
| PTH         | Organs          | % seeding |
| Control     | Bone marrow     | 43       | 34       |
|             | Spleen          | 5        | 7        |
| PTH, 10 µg/kg | Bone marrow     | 11       | 18       |
|             | Spleen          | 8        | 10       |
| PTH, 80 µg/kg | Bone marrow     | 9        | 39       |
|             | Spleen          | 6        | 1        |

**Methods.** The seeding efficiency (f-24) of short-term (CFU-S) and long term (CAFC-28) hematopoietic precursor cells to the stromal cells of bone marrow and spleen of lethally irradiated PTH treated recipients was measured 24 hours after intravenous transplantation. Mice were injected i.p. with PTH 80 µg/kg 5 days/week for 4 weeks. PTH treated and control mice were exposed to 12 Gy total body irradiation and than injected i.v. with 1×10⁶ bone marrow cells, previously subjected to two-hour adhesion to plastic. 24 hours later cells from spleen and pooled femur and tibia were harvested. To measure the content of CFU-S homed to the spleen of bone marrow secondary lethally irradiated recipients were injected with either 1/30 of spleen cells or 1/5 of pooled femur and tibia equivalent. CAFC-28 frequency was analyzed in the homed suspension by limiting dilution analysis with standard assay using MS-5 layers. Four dilution steps had been done, with the concentration being halved each time. The first concentration used was 1/24 of total spleen cell amount and 1/4 of pooled femur and tibia cells per well. Results. F-24 of short-term HSC in spleen was the same in treated and control mice, but decreased 3-fold in the bone marrow of PTH treated mice. For long-term HSC, F-24 in bone marrow was similar in both groups, whereas in spleen it decreased dramatically up to 7-fold. If femur and tibia represent 14% of total pool of murine bone marrow cells (Colvin, 2004) it is possible to calculate homing of HSC in PTH treated mice (see table). About 60% of CAFC-28 did not homed to this multivariate statistical analysis. Multivariate analysis confirmed the role of ACC genotype as a risk factor of more aggressive nHL manifestation (OR=3.57, p=0.05) in addition to the elevated LDH480 level (OR=3.95, p=0.04). Next analysis performed with respect to the number of extra nodal sites showed, although not highly significant (0.05<p<0.1), influence of the presence ofACC genotype. **Conclusion:** The low producer genotype of anti-inflammatory Th2 cytokine IL-10 was found to be associated with an unfavorable course of nHL.
0842

SPONTANEOUS TRANSFORMATION OF LYMPH NODE AND BONE MARROW STROMAL CELLS FROM CANCER PATIENTS

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Background. Until recently, human cells were regarded resistant to spontaneous in vitro transformation. Last year, two papers in Cancer Research and Cytotherapy reported that diploid mesenchymal stem cells (MSCs) can convert to malignant phenotype in vitro in presence of high concentration of fetal calf serum. AIMS. We have obtained our first transformed stromal cell line in 2003. Since then, we have been studying conditions necessary for spontaneous in vitro transformation and in vitro and in vivo properties of transformed stromal cells. METHODS. Lymph node stromal cells were obtained from patients undergoing diagnostic or curative surgical procedure for lymph node (4 patients) or epithelial cancer (5 patients). Bone marrow MSCs were obtained from patients undergoing diagnostic or staging bone marrow biopsy. After tissue disaggregation, cells were centrifuged on Ficoll gradient and mononuclear cells were allowed to adhere to tissue culture plastic. Adherent cells were grown in α-MEM with 10% fetal calf serum (FCS) or in α-MEM with 2% FCS supplemented with dexamethasone, ascorbic acid, EGF and PDGF-BB. Surface, cytoplasmatic and nuclear antigens were studied by flow cytometry and immunofluorescence. Cyto genetic analysis was performed after standard G-banding. Transformed cells were injected subcutaneously or intraperitoneally into sublethally irradiated NOD/LSz Rag-Insull mice and tumors were examined histologically. RESULTS. We have obtained transformed stromal cells from all lymph nodes grown in α-MEM with 10% FCS. Transformation occurred very quickly, during initial expansion in one case and from 5th to 10th passage in other cases. Only two transformed cell lines were obtained from more than twenty bone marrow aspirates and in both cases, the transformation occurred during 2nd passage. Before transformation, cell culture morphology was neither mesenchymal nor crisis phases and normal cells were very quickly overgrown by morphologically abnormal cells with average doubling time of 38 hours. Immunophenotypically, these cells resembled MSCs and were CD90+, CD166+, CD34-, CD45-, cytokeratin- and CD117+. They were also positive for telomerase, grew without contact inhibition and were unable to differentiate into osteoblasts or adipocytes. Transformed cells were hypodiploid to hypertetraploid (49-115 chromosomes), with nonrandom pattern of chromosomal gains and losses. When administered subcutaneously into immunodeficient animals, these cells produced locally invasive sarcomas and in several cases, visceral metastases were found after intraperitoneal implantation. On the other hand, cells from the same samples grown in α-MEM with 2% FCS only retained their usual spindle-shaped morphology, contact inhibition, diploid karyotype and ability to differentiate into specialized cells. Conclusions. Stromal cells from cancer patients lymph node were prone to quick malignant transformation, while mesenchymal stem cells from bone marrow were much more resistant. For transformation, growth medium with 10% FCS was required in both cell types. After transformation, all the cell lines had very similar phenotype, karyotype and clinical behaviour. Whether the easy in vitro transformation is an inherent feature of lymph node stromal cells or reflects the wide-spread genomic instability of cancer patients remains to be established.

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0843

DOWN REGULATION OF ACTIVIN A BY LYMPHOMA IN THE BONE MARROW: A POSSIBLE MECHANISM OF BONE MARROW INVOLVEMENT

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Background. Activin A is thought to potentiate cell survival in bone marrow biopsies; absence of significant tear drop poikilocytosis and leukoerythroblastosis on peripheral blood smear; normal sized spleen; positive autoimmune serology, possibly fulminating autoimmune myelofibrosis (AM); increased reticulin fibrosis, not clustered megakaryocytes, reactive lymphoid infiltration, increased reticulin fibrosis, not clustered megakaryocytes; reactive lymphoid infiltration. In our previous investigations, we have found that activin A is significantly down-regulated in the vicinity of pathological entity, resulting in various degrees of isolated or combined chronic peripheral blood cytopenias. It is defined by a pattern including: increased reticulin fibrosis, not clustered megakaryocytes, reactive lymphoid infiltration in bone marrow biopsies; absence of significant tear drop poikilocytosis and leukoerythroblastosis on peripheral blood smear; normal sized spleen; positive autoimmune serology, possibly fulfilling the classification criteria of an autoimmune disease. It has to be distinguished from different conditions associated with myelofibrosis; among these, the most relevant differential diagnosis is with chronic idiopathic myelofibrosis (CIM), particularly when disclosing autoimmune clinical and/or laboratory features. AIM. We purposed to assess the bone marrow stromal changes in AM with particular regard to the

Figure 1. Low power view of activin A in the bone marrow.

Methods. The patient population consisted of all consecutive patients diagnosed with lymphoma between the years 2000-2004. In accordance with the IRB of our hospital, paraffin embedded sections were prepared and immunohistochemical staining was performed using an antibody to activin A. The slides were reviewed by team of 5 investigators and graded separately. We analyzed 17 patients with lymphoma and 3 patients without lymphoma served as controls. Results. Out of 17 lymphoma cases, 10 patients showed BM involvement while 7 patients were without BM involvement. In the former group the level of activin A was significantly decreased in the area surrounding the lymphoid infiltrate (Figure 1A). This was seen uniformly in all the patients except for one, regardless of the original histology of the tumor (follicular or diffuse). The level of activin A in the rest of the BM was similar to the level seen in specimens of reactive BM. In all 7 patients who had no BM involvement we found a diffuse staining for activin A (Figure 1B) (similar to what we saw in patients with reactive BM). Conclusions. Lymphoid cells have the ability to migrate to the bone marrow. It is interesting therefore that only some of the patients with malignant lymphomas have BM involvement. This could stem from a difference in the migratory abilities of the lymphoid cells, which is unlikely, or from a difference in their ability to home and flourish in the BM microenvironment. We demonstrated that activin A is significantly down-regulated in the vicinity of the lymphoid ‘metastatic’ lymphoma, as opposed to what occurs in normal inflammatory BM. This suggests that an interaction between the lymphoma cell and the BM microenvironment leads to down-regulation of activin A expression and possibly promotes the survival of the lymphoid cells.

0844

EVALUATION OF THE EXPRESSION OF ANGIogenic CYTOKINES AND THEIR RECEPTORS IN AUTOIMMUNE MYELOFIBROSIS

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Background. Autoimmune myelofibrosis (AM) is an emerging clinicopathological entity, resulting in various degrees of isolated or combined chronic peripheral blood cytopenias. It is defined by a pattern including: increased reticulin fibrosis, not clustered megakaryocytes, reactive lymphoid infiltration in bone marrow biopsies; absence of significant tear drop poikilocytosis and leukoerythroblastosis on peripheral blood smear; normal sized spleen; positive autoimmune serology, possibly fulfilling the classification criteria of an autoimmune disease. It has to be distinguished from different conditions associated with myelofibrosis; among these, the most relevant differential diagnosis is with chronic idiopathic myelofibrosis (CIM), particularly when disclosing autoimmune clinical and/or laboratory features. AIM. We purposed to assess the bone marrow stromal changes in AM with particular regard to the
expression of angiogenic cytokines and their receptors, estimation of microvessel density (MVD), and immunophenotype of the lymphoid component. The aim of the present study was to evaluate, by immunohistochemistry, the expression of various isoforms of angiogenic cytokines and their receptors in bone marrow biopsies of AM in comparison with the expression patterns of the same cytokines and their receptor expression in CIM and normal bone marrows, as described by Chou et al. (Leuk Res 2003; 27: 499) and Yoon et al. (Acta Haematol 2000; 104: 151), respectively. Methods. The tested cytokines and their receptors included platelet derived growth factor (PDGF, PDG-Fα), basic fibroblast growth factor (bFGF) and its receptors (FGFR1, FGFR2, FGFR3, FGFR4), vessel endothelial growth factor (VEGF) and its receptors (VEGFR1, VEGFR2, VEGFR3), TGF-β1, TGF-β2, TGF-β3 and its receptors (TGFβRI, TGFβRII). Immunohistochemistry was performed by an immunoperoxidase method with avidin-biotin complex, using specific commercial antibodies (Santa Cruz Biotechnology, USA) on trephine biopsies derived, before treatment, from eight patients (age range: 48-78 years; 6 females) diagnosed as affected by AM. Controls skipping primary antibodies were used as negative controls. Results. The immunohistochemical staining for TGF_R1 on endothelial cells of small vessels, and bFGF on megakaryocytes were markedly decreased compared to those observed in CIM samples. For the other tested cytokines and their receptors, AM samples showed patterns of staining intensity and cellular distribution similar to those found in CIM and normal bone marrows. Conclusions. The results of our comparative study suggest that the different bone marrow expression of TGFβRI in endothelial cells and bFGF in megakaryocytes could be useful to differentiate AM from CIM.

0845 PROINFLAMMATORY CYTOKINES IN THE PATHOGENESIS OF DENGUE FEVER AND HEMORRHAGIC DENGUE FEVER IN VENEZUELA

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Background. Dengue fever is an flu-like acute viral disease highly prevalent in several regions of Asia and America. It is transmitted by the mosquito Aedes aegypti. In around 30% of the patients, the disease progresses towards the haemorrhagic form, which may be potentially life-threatening when haemorrhagic shock develops. The disease has become endemic in Venezuela, constituting a severe problem of public health. The pathogenesis of the haemorrhagic form of the disease is far from clear, although several cytokines are believed to play an important role. MATERIALS AND METHODS. During the period 2004-2006, especially in the rainy season, numerous cases of dengue fever (DF) were detected in Venezuela and studied severly two (2) patients, who were tested 168.8 ± 77.4 pg/ml to 49 ± 45.2 (10 days), 167.8 ± 67.2 (17 days) pg/ml, IL-12 Mix; mean ± SD, from 222.6 ± 95.3 pg/ml to 48.6 ± 44.1 (10 days), 226.6 ± 98.2 (17 days) pg/ml at 10 days and 17 days after chemotherapy (IL-12 p40; p=0.011 (10 days), p=0.006 (17 days), IL-12 Mix; p=0.0011 (10 days), p=0.0011 (17 days)). These results showed that administration of G-CSF decreased serum IL-12 p40 and IL-12 Mix levels. Interestingly, plasma IL-12 p40 level in CG group patients with clinical stages was and was not significantly decreased after chemotherapy than before chemotherapy (mean±SD, -95.6±131.1 pg/ml) (n=16 course) compared with group C (mean±SD, -0.1±35.2 pg/ml) (n=10 course) (p=0.035). However, plasma IL-12 Mix level in CG group patients with clinical stages and was not significantly decreased after chemotherapy than before chemotherapy. Plasma IL-12 p70 levels could not be detected in almost all patients. We analyzed the association with survival rate after chemotherapy (OS) at 24 months was not significantly differed between both groups (58.3% VS GC 80.0%, p=0.67). However, the survival in the patients of clinical stages and with CG group (n=6) significantly improved than C group (n=4) (stages and survival rate 66.6% vs 25.0%, p=0.02). Conclusions. We found that chemotherapy with G-CSF decreased IL-12 p40 production. We did not find the difference in overall survival at the present time, however, a longer observation with G-CSF appears to influence in overall survival rate by reducing an immunosuppressive IL-12 p40 production.
of the current study was to measure the levels of circulating angiogenic molecules in Hodgkin's patients prior to and after treatment and correlate them to disease stage and prognostic score. Patients-Methods. Serum samples were obtained from sixty patients with newly diagnosed Hodgkin's disease (mean age±SD: 41±19 years) and nineteen healthy individuals (mean age: 59±10 years). Serum samples were obtained from all patients prior to initiation of treatment and in 48 within 6 months of completion of standard ABVD therapy. Six patients relapsed in less than 6 months and 5 died. Two of the 60 patients were diagnosed as Hodgkin's Ann Abor's stage I, 42 stage II, 5 stage III, 10 stage IV. International Prognostic Scores (IPS) of 0, 1, 2, 3, 4 and 5 were recorded for 14, 15, 18, 5, 1 and 3 patients. Elisa measurements were performed using the Quantikine, B&D kits (Minneapolis, MN, USA) for human Hepatocyte growth factor (HGF), Vascular endothelial growth factor (VEGF), Angiogenin, Angiopoietin-2, tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6). Results. Using the non-parametric Mann-Whitney test, there was strong evidence of higher median concentrations in the pre-treatment group compared to controls for TNF-α (20.8 versus 14.9 pg/ml, p<0.001), HGF (1958.8 versus 744.1 pg/ml, p<0.001), Angiogenin and angiopoietin-2 levels did not differ from controls. TNF and HGF were found increased in stages III/IV in comparison to stages I/II (p<0.001). No statistically significant differences between patients with low and high prognostic score were detected. HGF and VEGF correlated significantly with IL-6 (r=0.56, p<0.0001) and VEGF (794.4 versus 297.4 pg/ml, p=0.001). Angiogenin and angiopoietin-2 levels did not differ from controls. TNF and HGF were found increased in stages III/IV in comparison to stages I/II (p<0.001). No statistically significant differences between patients with low and high prognostic score were detected. HGF and VEGF correlated significantly with IL-6 (r=0.56, p<0.0005 and r=0.57, p<0.001 respectively), HGF, TNF-α, VEGF and angiogenin decreased significantly following effective treatment (p<0.01). Conclusion. In conclusion, Hodgkin's disease displays an angiogenic activity as depicted by the increased serum levels of a number of angiogenic cytokines. HGF seems to be the prominent molecule in Hodgkin's disease, which may be used to monitor the disease status and the response to treatment.

0848 LEVELS OF CYTOKINES AND OTHER INFLAMMATORY MARKERS IN PATIENTS AFTER ALLOGENIC STEM CELL TRANSPLANTATION

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Objective: Infectious complications remain a major cause of morbidity and mortality after allogeneic haematopoietic stem cell transplantation (HSCT). Diagnosis and outcome might be improved by using early, sensitive and specific laboratory parameters. The aim of the study was observation of dynamics for some inflammatory markers: IFN-γ, TNF-α interleukin (IL)-18, IL-8, serum amyloid A (SAA), C-reactive protein (CRP), procalcitonin (PCT) and neopterin measured at the completion of standard ABVD therapy. Patients-Methods. We studied 20 patients (mean age 32.4 ± 1.0 years) with haematological malignancies and aplastic anaemia undergoing allogeneic HSCT from related donors with different sensitivity to proinflammatory cytokines. Protective mechanisms depended on the stage of erythroid differentiation. Results. Major transplant-related complications (MTCs) included bakteriemia, veno-occlusive disease of the liver, idiopathic pneumonia syndrome, CMV infection, endothelial leakage syndrome and grade ≥ II acute graft-versus-host disease (GVHD). The effect resulted significantly higher than that due to signalling inactivated by death receptors, was found diminished in K562 induced to differentiation by haemin were induced to apoptosis. This effect resulted significantly higher than that due to signalling inactivation of the growth factor erythropoietin via PI3Kinase (Apolipoproteins: H-Ly-T 92.2 ± 2.4% vs. H-Ly 68.9 ± 5.3%, p<0.01). Results of caspase-3 activity measured at 6 h-incubation of cell lysates with chromogenic substrate parallel those of apoptotic K562 cells (Figure B). mRNA levels of Bcl-x, the Bcl-2 related protein that acts as important regulator of cell death, were not modified under the experimental conditions mentioned above. The mRNA of c-FLIP, the suppressor protein of apoptotic signals induced by death receptors, was found diminished in K562 induced to erythroid differentiation but not in UT-7 cells grown under similar conditions. Conclusions. During the process of differentiation, cells become sensitive to proapoptotic action of TNF-α. A decrease in c-FLIP expression would explain the apoptosis produced by TNF-α in K562 cells induced to differentiation since this cytokine effect was not observed in differentiated UT-7 cells with non-altered mRNA c-FLIP levels. Besides, cells with different dependence on the growth factor erythropoietin, analysed under similar conditions of erythroid differentiation, show different sensitivity to proinflammatory cytokines. Protective mechanisms against cellular apoptosis caused by TNF-α seem to be mediated by PI3Kinase signalling and proved to be independent from Bcl-x. These findings may have potential implications in the understanding of the mechanisms underlying anaemia in chronic inflammatory diseases.

0849 TNF-α INDUCES APOPTOSIS IN CELLS UNDER ERYTHROID DIFFERENTIATION

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Backgrounds. Inflammatory cytokines inhibit the proliferation of erythroid progenitor cells, among other effects upon iron homeostasis and erythropoietin synthesis, all of which contribute to the pathogenesis of anaemia, a common complication of chronic diseases. Recent studies suggest that patients increased release of TNF-α could be responsible for the development of anaemia through the induction of an apoptotic mechanism mediated by death receptors. It has been suggested that this cytokine effect is dependent on the stage of erythroid differentiation. Aim. The effect of TNF-α upon differentiation, proliferation and apoptosis was investigated in cells subjected to erythroid differentiation. Methods. K562 (erythropoietin-independent) and UT-7 (erythropoietin-dependent) cells were cultured in the presence of haematin (H) for 48 h to study proliferation (Trypan blue test), differentiation (DAF staining) and apoptosis evaluated by apoptotic cells (Hoechst fluorescent nuclear stain) and caspase-3 activity (proteolitic cleavage of chromogenic substrate). mRNA analysis was performed by RT-PCR. Results. After 48 h with H, high levels of haemoglobinized cells were observed (K562: 85%, UT-7: 78%). On the other hand, 30 ng/ml TNF-α treatment did not induce significant changes in the development, maturation and viability of both cell lines. Non-differentiated K562 cells were not affected by TNF-α (T) whereas haematin-treated cells were sensitive to the TNF-α proapoptotic effect (Figure A: H-T vs. H, p<0.0002). This apoptotic action was enhanced by PI3Kinase inhibition with Ly294002 (Fig. A. H-Ly-T vs. H-Ly, p<0.05). The negative effects observed in the presence of TNF-α were dramatically decreased by a previous treatment with anti-TNF neutralizing antibody (Figure A). Only in simultaneous experiments with TNF-α and ly, UT-7 cells cultured in the presence of erythropoietin and induced to differentiation by haematin were induced to apoptosis. This effect resulted significantly higher than that due to signalling inactivation of the growth factor erythropoietin via PI3Kinase (Apolipoproteins: H-Ly-T 92.2 ± 2.4% vs. H-Ly 68.9 ± 5.3%, p<0.01). Results of caspase-3 activity measured at 6 h-incubation of cell lysates with chromogenic substrate parallel those of apoptotic K562 cells (Figure B). mRNA levels of Bcl-x, the Bcl-2 related protein that acts as important regulator of cell death, were not modified under the experimental conditions mentioned above. The mRNA of c-FLIP, the suppressor protein of apoptotic signals induced by death receptors, was found diminished in K562 induced to erythroid differentiation but not in UT-7 cells grown under similar conditions. Conclusions. During the process of differentiation, cells become sensitive to proapoptotic action of TNF-α. A decrease in c-FLIP expression would explain the apoptosis produced by TNF-α in K562 cells induced to differentiation since this cytokine effect was not observed in differentiated UT-7 cells with non-altered mRNA c-FLIP levels. Besides, cells with different dependence on the growth factor erythropoietin, analysed under similar conditions of erythroid differentiation, show different sensitivity to proinflammatory cytokines. Protective mechanisms against cellular apoptosis caused by TNF-α seem to be mediated by PI3Kinase signalling and proved to be independent from Bcl-x. These findings may have potential implications in the understanding of the mechanisms underlying anaemia in chronic inflammatory diseases.
0850
DEVELOPMENT OF MALIGNANCIES IN MICE TREATED WITH G-CSF FOR A LONG TIME
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Backgrounds. G-CSF is well recognized as a potent mobiliser of hematopoietic stem cells from the bone marrow into the blood, and is being accepted as a regulator of immune responses also. During recent years the use of peripheral blood instead of bone marrow as a source of stem cells has been increasingly employed in the allogeneic transplant setting. Although several experience concerning donors treated with G-CSF for stem cells mobilization proved it as a safe procedure. However, the parameters used for the safety evaluation have been rather crude. Few cases disclosing several morphological and cytogenetic changes in hematopoietic cells as well as temporal deregulation of some genes in healthy donors were published. Moreover the immunological complications and leukemia development were noted in G-CSF treated donors.

Aims. The goal of this investigation was to study the consequences of several courses of G-CSF treatment using low non-mobilising doses on mice model. Methods Female mice (CBAXC57Bl6) F1 and (DBA/2xBalb/c) F1 12-16 weeks old were injected subcutaneously with G-CSF (25 mcg/kg) for 4 days once a month with blood cell count and cytology measured before and after the course, another group of mice of the same strains were injected with G-CSF (5 mcg/kg) for 20 days per month with blood cell count and cytology measured monthly. G-CSF courses have been repeated monthly for half a year. After the termination of treatment blood cell count and cytology were performed in all groups. Total observation time was 21 months. The survival rate was evaluated in experimental and control groups. Results During 20 months of follow up 24 out of 40 G-CSF treated mice died due to unknown cause, 8 mice developed different hematopoietic or other malignancies and disorders and were sacrificed. All control mice were healthy with stable number of leukocytes and hemogram. Four-day treatment with 25 mcg/kg/d of G-CSF didn’t change the number of leukocytes significantly, while in the group treated for 20 days with a 5 mcg/kg/d the number of leukocytes had slightly increased. Most of the experimental animals had considerable reticulocytosis. Within the first 7 months of follow up among detected disorders myeloproliferative disorders had dominated, afterwards solid tumors were also detected. Most of animals became neutropenic before disease manifestation. All mice injected with G-CSF for 20 days a month developed pus inflammations in site of injection after 3 months of treatment. Summary/Conclusion. Long-term treatment of animals with low doses of G-CSF (total 100 mcg/kg/mo) total observation period was 21 months may induce malignant transformation and leads to significant decrease of life-span. Mobilization of hematopoietic stem cells with G-CSF is known to promote deregulation of several genes expression which returns to normal profile within 2 months. Perhaps prolonged administration of this growth factor leads to dramatic alterations in gene regulation once a month should be taken into consideration. Without G-CSF treatment or mobilization of hematopoietic stem cells in healthy volunteer individuals.

0851
PALIFERMIN IN PATIENTS WITH HEMATOLOGIC MALIGNANCIES UNDERGOING AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION (AUTO-HSCT). PRELIMINARY RESULTS
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Background and Aims. Oral mucositis (OM) is a frequent complication of myeloablative therapy and HSCT with no effective treatment. In this multi-center study we tested the ability of palifermin (recombinant human keratinocyte growth factor) to reduce the incidence, duration and severity of OM induced by high-dose chemotherapy followed by auto-HSCT in patients with hematologic malignancies. We also evaluated the requirement for analgesics and parenteral nutrition administered because of OM, incidence of febrile neutropenia (≥38°C), severe infections and requirement for additional antibiotics. Moreover, the influence of palifermin on the hematopoietic recovery after autoHSCT was assessed in this study.

Methods and Results. Fifty-six patients with hematologic malignancies were enrolled to the study. Twenty-eight of them (50%) received palifermin (60 microg/kg/day) for three consecutive days before and three consecutive days after conditioning therapy. The median age of the palifermin and control group was 38.3 (range, 19 to 58) and 37.9 (range, 19 to 64), respectively. OM was assessed daily after autoHSCT according to the World Health Organization (WHO) scale. The incidence of OM of WHO grade 4 was 42.9% and 25.8% in the palifermin group and 96.4% in the control group and grade 3-4 was 3.5% in the palifermin group and 32.1% in the control group. Among all patients the median duration of OM was 2.9 days (range, 0 to 11) in the palifermin group and 5.1 days (range, 0 to 27) in the control group. As compared with control, palifermin was also associated with significant reductions in the use of analgesics (21.4% vs. 71.4%, p < 0.001), opioid analgesics (10.7% vs. 53.3%) and parenteral nutrition (8.5% vs. 28.5%; p < 0.001). There were no significant differences in the incidence of febrile neutropenia, severe infections and requirement for additional antibiotics observed between groups. Also palifermin did not impaired reconstitution of the hematopoietic system (Table 1).

The drug was generally well tolerated. Adverse events, mainly rash, pruritus, erythema, generalized oedema, mouth/tongue thickness and discoloration, taste alteration and proteinuria were mild to moderate in severity and were transient. Conclusions. Palifermin administration significantly reduced the incidence, severity and duration of OM and did not have negative effect on engraftment in the patients with hematologic malignancies after autoHSCT.

Table 1.

<table>
<thead>
<tr>
<th>ANC 0.5×10⁹/L</th>
<th>Platelets 20×10⁹/L</th>
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<tr>
<td>Palifermin group</td>
<td>Median (days) range</td>
</tr>
<tr>
<td></td>
<td>15.8 9 to 42</td>
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<td></td>
<td>16.3 8 to 45</td>
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0852
EFFECTIVE MOBILIZATION BY PEG-FILGRASTIM PLUS ARA-C CONTAINING REGIMEN IN PRETREATED LYMPHOMAS PATIENTS
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Background. Studies performed on mice and healthy human volunteers have shown that a single dose of pegfilgrastim (Peg-GCSF) is effective in stimulating peripheral blood stem cells (PBSC) mobilization. Aims. The aim of this study was to evaluate the efficacy of pegfilgrastim, in combination with salvage chemotherapy, in mobilizing CD34(+) stem cells into the peripheral blood of pretreated lymphoma patients.

Methods: We studied 27 pretreated patients (Hodgkin’s lymphomas=5; non-Hodgkin’s lymphomas=22). The median age was 57 years (range 17-70). The patients received a median of 2 previous chemotherapy regimens. Median time from mobilization to harvest was 11.5 days. The efficacy of the mobilizing procedure was tested in lymphoma patients receiving salvage regimen [DHAP (cisplatin 100 mg/m², cytarabine 2000 mg/m² × 2) in 17 or MAD (cytarabine 2000 mg/m² × 2 × 5 days in 10) plus pegfilgrastim in 11 or filgrastim in 16. Pegfilgrastim was given as single subcutaneous injection (6 mg) on day +5 post chemotherapy. Filgrastim was given daily (10 μg/Kg) from day +5. Daily monitoring of circulating CD34+ cells was started from day 8 after the end of chemotherapy. Results. Twenty-five/27 patients reached the target cell dose of 2.5 × 10⁶ cells/kg. A median of 2 apheresis (range 1-4) was performed. In pegfilgrastim group, a median of 5.27 × 10⁶ CD34+ cells/kg (range 1.06-10) was collected. in filgrastim group, a median of 11 × 10⁶...
CD34(+)- cells/kg (range 0.09-32.84) was collected. No statistical difference (p=0.06) between the two groups (Pegfilgrastim vs. Filgrastim) was found (Table 1). Conclusions. Our results show that pegfilgrastim as an adjunct to Hidracet based chemotherapy is an effective mobilization regimen in pretreated lymphoma patients also effective as filgrastim based regimen. This approach is to be confirmed in larger series of patients and probably with increased dose of pegfilgrastim and could open new opportunities in stem cell mobilization for poor or non-mobilizers patients with malignant lymphomas.

Table 1. Pegfilgrastim vs Filgrastim in 27 pts (51 apheresic procedures).

<table>
<thead>
<tr>
<th>Pegfilgrastim</th>
<th>Filgrastim</th>
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<tr>
<td>N° apheretic procedures</td>
<td>2.1</td>
</tr>
<tr>
<td>Median day to 1st harvest</td>
<td>13.5 (+11-16)</td>
</tr>
<tr>
<td>Median CD 34x10^6 (overall 8,79)</td>
<td>4.96</td>
</tr>
<tr>
<td>Poor mobilizer (&lt; 2.5 CD 34x10^4) overall 6 pts (22%)</td>
<td>4</td>
</tr>
<tr>
<td>Very poor mobilizer (&lt; 1 CD 34x10^3) overall 3 pts (11%)</td>
<td>1</td>
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(>2,5 CD 34x10^4) overall 21 pts (78%).

0853

LOW DOSE LENOGRASTIM IS AS EFFECTIVE AS STANDARD DOSE IN SHORTENING NEUTROPHIL ENGRAFTMENT TIME FOLLOWING MYELOABLATIVE CHEMOTHERAPY AND PERIPHERAL BLOOD PROGENITOR CELL RESCUE

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Backgrounds. G-CSF is widely used following HDT and PBPCR to reduce neutrophil engraftment time. The dose and duration required to gain maximum clinical and economic benefit has not been fully investigated. Aims. This double blind placebo-controlled randomised trial was performed to determine whether short course low-dose or standard-dose L would influence recovery of haematopoiesis following HDT and PBPCR. Methods. 61 patients (pts) with non-Hodgkin lymphoma (40) or Hodgkin lymphoma (21) undergoing HDT were randomised between May 1999 and November 2004. Pts had normal peripheral blood counts prior to HDT (Hb ≥100g/L, total white cell count ≥3.0x10^9/L, neutrophils (N) ≥1.0x10^9/L and platelets ≥50x10^9/L), and had a minimum 2.5x10^9/L CD34+ cells/kg PBFC previously collected following mobilisation with Cyclophosphamide 3 g/m2 and G-CSF. All received HDT with BCNU 300 mg/m^2 d-7, Etoposide 200 mg/m^2 od s-5-d-2, Cytosine arabinoside 200 mg/m^2 bd d-5-d-2 and Melphalan 140 mg/m^2 d-1 before return of PBPC on d0. Pts were allocated standard dose L 265 µg daily (20 pts), low dose L 105 mcg daily (21 pts) or placebo injections (20 pts). These commenced on day +5 following PBPCR and continued until Nso 0.5x10^9/L. Pts received standard supportive care including prophylactic Fluconazole and Acyclovir, but not routine antibacterial prophylaxis, until haemopoietic recovery. Results. L at any dose resulted in a significantly shorter median time to N recovery ≤10 (10.0 vs 11.0 days, p=0.02) and ≥10 (11.0 vs 14.0 days, p=0.0065) compared to placebo. The only significant difference between standard- and low-dose L was in hospital stay (21.0 vs 22.0 days, p=0.04), however L at any dose showed a significant reduction over placebo (22.0 vs 23.0 days, p=0.01). There was no significant difference in blood product support or antibiotic usage between the groups. At a median follow up of 40 months there were 27 confirmed lymphoma relapses and 26 deaths (21 relapsed lymphoma, 1 secondary AML, 4 other). Conclusions. Short course low dose L is as effective as standard dose in reducing neutrophil engraftment time following HDT and PBSCR. L at any dose reduces hospital stay when compared to placebo. This approach should be considered for those patients in whom growth factor support is indicated.

0854

MODULATION OF PROTEIN TYROSINE PHOSPHATASE 1B BY ERYTHROPOIETIN IN UT-7 CELL LINE

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Backgrounds. The central role played by tyrosine phosphorylation of erythropoietin receptor (EpoR) in cell activation by erythropoietin (Epo) has focused attention on protein tyrosine phosphatases (PTP) as candidates implicated in the pathogenesis of the resistance to therapy with human recombinant Epo. The prototypic member of the PTP family is PTP1B, a widely expressed non-receptor PTP located both in cytosol and intracellular membranes via its hydrophobic C-terminal targeting sequence. PTP1B has been implicated in the regulation of a number of signaling pathways, in particular, those involving tyrosine phosphorylation induced by growth factors, cytokines and hormones such as the down-regulation of EpoR and insulin receptor. Binding of ligand to cell-surface EpoR results in the activation of JAK2 and phosphorylation of tyrosine residues in the cytosolic domain of the receptor. Termination of the EpoR signaling is attributed to the cytosolic SH-PTP1B. However, it has been demonstrated that PTP1B also participates in down-regulation of the ligand-activated cell surface EpoR. Aim. To investigate the effect of Epo on PTP1B expression. Methods. The UT-7 human cell line was used as an Epo-dependent model. Epo was added to serum- and Epo-deprived cells for previous 18 h. After different periods of Epo incubation, cells were lysed and total proteins and RNA were obtained. cDNA was prepared from different RNA samples and PTP1B mRNA level was analysed by Real Time PCR. Total proteins or immunoprecipitates with anti-PTP1B were subjected to Western Blot using anti-PTP1B or anti-PTyr. Immunoprecipitates were also subjected to a PTP1B activity assay with pNPP. Then, the experiment was repeated including pretreatment with LY294002 (PI3K inhibitor) before Epo stimulation. Results. An increased and maximum level of PTP1B mRNA was already observed at 3 h of Epo stimulation (figure a). This increment correlates with the induction of PTP1B expression observed by Western Blot (figure b). However, after 9h with Epo, mRNA level returned to baseline while protein expression remained constant. PTP1B Tyr phosphorylation was detectable after 5 min of Epo stimulation and declined within 6 h PTP1B activity increased after 3 h of Epo incubation and diminished to the basal level within 6 h (figure C). Figure d shows that the pretreatment of UT-7 cells with LY294002 downregulated PTP1B expression in a dose-dependent manner reaching the highest inhibition at 100 mM LY concentration. Conclusions. We have found an Epo-induced expression of PTP1B, associated with increased PTP1B Tyr phosphorylation, suggesting that besides modulating Epo/EpoR signaling, PTP1B suffers a feedback regulation by Epo.
PLASMA CYTOKINE PROFILE IN HEALTHY INFANTS IS NOT INFLUENCED BY VACCINATION

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Objectives. The purpose of this study was to investigate whether the plasma cytokine profile in healthy infants is not influenced by vaccination.

Background. The plasma cytokine profile in healthy infants is not influenced by vaccination. This study is important for the understanding of the immune system of infants and the role of cytokines in the development of the immune system.

Methods. A total of 100 healthy infants were included in the study. Blood samples were obtained before and after vaccination with the combination vaccine (DTaP, hepatitis B, polio, and IPV). The cytokine levels were determined using a multiplex bead assay.

Results. No significant differences were found in the plasma cytokine levels before and after vaccination. The cytokines studied were IL-1β, IL-2, IL-4, IL-6, IL-10, IFN-γ, and TNF-α.

Conclusions. The plasma cytokine profile in healthy infants is not influenced by vaccination. This study provides important information for the understanding of the immune system of infants and the role of cytokines in the development of the immune system.

References

MONOCYTE CHEMOTRACTANT PROTEIN-1 AND INTERLEUKIN-8 LEVELS IN ACUTE INFLAMMATION INDUCED BY PROLONGED BRISK EXERCISE: CLINICAL IMPLICATIONS

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Monocyte chemotactic protein-1 (MCP-1), also known as CCL2, a chemokine that regulates migration and infiltration by monocytes/macrophages, belongs to the CC chemokine subfamily. Interleukin-8 (IL-8), also known as CXCL8, a proinflammatory chemokine with angiogenesis-promoting properties belongs to the CXC chemokine superfamily. Both MCP-1 and IL-8 have important roles in the pathogenesis of many chronic inflammatory disorders, including atherosclerosis and obesity. Their proinflammatory effects are mediated mainly by the CC chemokine receptor 2 (CCR2) and CXCR1/2, respectively. MCP-1 and IL-8 cause chronic vascular inflammation and induce thrombosis, proliferation and migration of vascular smooth muscle cells, angiogenesis, and oxidative stress. Previous studies indicate that: 1) MCP-1 production from endothelial cells, smooth muscle cells, and regional leukocytes increases in the presence of endothelial dysfunction and atherosclerosis risk factors; 2) MCP-1 and IL-8 expression is increased in atherosclerotic lesions and injured arteries; and 3) eliminating MCP-1 function decreases neointimal hyperplasia after injury and atheroma formation in mice. We studied the association between MCP-1 and IL-8 levels and the degree of inflammation in 15 athletes that participated in the ultra-distance foot race of the 246 km ‘Spartathlon’. This race consists of continuous, prolonged, brisk exercise. We reported earlier that Interleukin-6 (IL-6), a proinflammatory chemokine (CRP), Serum amyloid A protein (SAA) and free plasma DNA levels markedly increased (by 8000-15208- and 10-fold, respectively) over the baseline at the end of the race. However, IL-6 levels returned to normal by 48h, while CRP, SAA and free plasma DNA remained elevated. Circulated levels of MCP-1 and IL-8 were measured by means of a multi-analyte Biochip Array Technology, using the Evidence analyzer (Randox Laboratories, UK). The measurements were performed before (phase I), at the end (phase II) and 48h post-race (phase III). MCP-1 levels at phase I (216.9 ± 48.5 ng/L) increased significantly at phase II (592.9 ± 115.7 ng/L) and subsequently decreased at phase III (278.1 ± 62.7 ng/L). At the same time period, IL-8 followed a similar pattern (phase I: 9.4 ± 4.5 ng/L, phase II: 28.5 ± 8.8 and phase III: 8.9 ± 4.3 ng/L). A significant positive correlation between MCP-1 and IL-8 was found at phase III (r = 0.345, p < 0.01), while this correlation was absent in the other two phases, indicating an independent response of each chemokine to inflammatory stimuli in each athlete. In conclusion, prolonged exercise induces an inflammatory response that is expressed by an increase in circulating MCP-1 and IL-8 levels. Whether these changes have long-term negative effects on the vasculature remains unknown.

References

EVALUATION OF THE EFFECTS OF THE CD33-TARGETED DRUG GEMTUZUMAB OZOGAMICIN ON GROWTH AND HISTAMINE RELEASE IN HUMAN MAST CELLS AND BASOPHILS

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Objectives. Mylotarg (gemtuzumab-ozogamicin–GO) has recently been introduced as a novel CD33-targeting drug in clinical hematological. However, despite clinical efficacy in acute myeloid leukemia, GO produces significant side effects including an infusion-syndrome. We have recently shown that mast cells (MC) and basophils (BA) express CD33. In the present study, we investigated the effects of GO on mediator secretion and growth of MC and BA. Methods. Growth-inhibitory effects of GO on neoplastic MC (HMC-1) and BA (KU812) as well as cord blood-derived MC- and BA progenitor cells were determined by counting cell numbers and the numbers of apoptotic cells. The amount of histamine secreted from primary MC and BA were measured after incubation of cells with GO alone or GO together with an anti-IgE antibody. Results. MC and BA as well as HMC-1 cells and KU812 cells were found to express CD33 mRNA and the CD33 protein. GO was found to inhibit the growth of HMC-1 cells and KU812 cells as well as SCF-dependent differentiation of MC and IL-3-induced growth of BA from

References
their cord blood-derived progenitors. The GO-induced inhibition of growth of neoplastic cells was found to be associated with induction of apoptosis. GO neither induced secretion of histamine from MC or BA nor did GO upregulate the anti-IgE-induced release of histamine in these cells. Conclusions. GO counteracts cell growth in normal and neoplastic human MC and BA without inducing release of histamine. Therefore, GO may be considered as a new targeted drug for the treatment of high-grade MC- and BA neoplasms.

0859
ALTERATIONS IN GENE EXPRESSION IN MURINE LEUKEMIA CELLS DEVELOPED AFTER G-CSF TREATMENT
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Background. Four-day treatment of mice with low (25mcg/kg) G-CSF doses is known to be insufficient for mobilization of hematopoietic stem cells into peripheral blood almost halves the content of bone marrow primitive hematopoietic stem cells and doesn’t affect the CFU-S number. Female mice (CBAxC57Bl6) F1 12-16 weeks old were subjected to such a course once a month. MFD-like myeloid leukemia with histiocytic sarcoma occurred in one case after 5rd course of G-CSF treatment. Liver tissue was totally substituted by undifferentiated cells with no morphologically definable features. The liver was about 4-5-fold enlarged by sight. Bone marrow and liver cells of the mouse were fully transplantable, recipients became moribund within 17-32 days since cells injection. All ill animals had enlarged liver (M 3.4 ± 0.5 g versus normal 1.38 ± 0.3 g). The developed leukemia was not of virus origin, which was proved by three independent methods. Aims. To understand molecular regulation of malignization, differentially expressed genes of interest must be identified, cloned and studied in detail. Methods. Subtract ed cDNA library from bone marrow cells of normal and leukemic mice was prepared by Suppression Subtractive Hybridization (SSH) method. Several up-regulated genes were further studied by RT-PCR in bone marrow and liver of leukemic mice. Normalization factor was evaluated by 3 housekeeping genes (HPRT1, RPL13A, UBC) by Genom software. Results. The clinically ill mice showed a moderate extent of anemia and reticulocytosis, which was supported by suppressed b-globin expression (top 5 down-regulated genes turned out to be b-globin genes). The expression level of c-abl and G-CSF doubled in bone marrow of leukemic mice compared with the normal bone marrow, while the concentration of CFU-C per 105 cells increased 4-fold (247 ± 31,2 in ill mice versus 57,9 ± 27,0 in control animals, p<0.01). The expression level of genes regulating cell proliferation did not change dramatically - only C-Myc expression increased 3-fold, however concentration of early hematopoietic precursor cells (LTC-IC) decreased about 5-fold (0.75 versus 3,32 per 105 cells in healthy mice). The pronounced changes were revealed in expression of MPO gene (3,4-fold increase). The liver of ill mice consisted of undifferentiated cells. As CD45 expression increased up to 11-fold simultaneously with constitution of liver parenchyma by tumor cell, one may suggest hematopoietic origin of invading cells. CFU-C were also revealed in affected liver (52,5 ± 7,7 per 105 cells). There were minor changes in G-CSF expression in liver cells of leukemic mice, whereas expression of G-CSF-R increased 18-fold compared with normal liver cells. Expression of c-abl also increased. Expression of anti-apoptotic genes was elevated up to 4-fold for bcl-2 and 2-fold for cIAP2. Unlike in the bone marrow, expression of JunB in the liver increased 5-fold. Summary. The G-CSF treatment may lead to development of myeloid leukemia with dramatically changed gene expression and high ability to invade liver tissue.

0860
A SINGLE FIXED DOSE INJECTION OF PEGFILGRASTIM TO MOBILISE AUTOLOGOUS STEM CELLS OF EXTENSIVELY PRE-TREATED LYMPHOMA AND MYELOMA PATIENTS; NOT ALWAYS SUCCESSFUL
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Backgrounds. In patients with multiple myeloma and refractory or relapsed lymphoma consolidation high-dose chemotherapy combined with stem cell rescue is an established therapy in chemosensitive disease. A commonly used approach to mobilise CD34 positive hematopoietic stem cells into the blood is the administration of granulocyte colony-stimulating factor following a course of chemotherapy. Pegfilgrastim, the pegylated form of filgrastim, is subject to a distinct method of clearance by neutrophilic leukocytes compared to filgrastim. Pegfilgrastim showed in earlier series of patients to be effective in mobilising blood progenitor cells in single fixed doses of 6 mg, as well as 12 mg. The optimal dose and scheduling of the injection and apheresis is not completely established in various patients groups with diverse extents of chemotherapeutical and radiotherapeutical pre-treatment. Aims. The primary aim was to study the feasibility of a single low dose of pegfilgrastim in extensively pre-treated patients. Methods. Forty-six Consecutive patients with myeloma or relapsed/refractory lymphoma who underwent stem cell mobilisation by identical apheresis techniques using either filgrastim or pegfilgrastim were retrospectively studied. Patient- and disease characteristics, pre-treatment data and mobilising chemotherapy type as well as cytokine dose, apheresis results and neutrophil recovery data were compared. Results. Stem cell harvest was performed in 24 patients after administration of pegfilgrastim once (6 mg) and in 22 patients after filgrastim. The filgrastim was administered in a median total dose of 4.2 mgs. (7.7 mgr./kg/day) and 11 injections were needed. The apheresis took place after 13 and 13.5 days respectively, with a maximum CD34-count of 8x10^6/L (pegfilgrastim group) and 11,6x10^6/L (filgrastim group). Of 24 patients who received pegfilgrastim, 5 patients showed a failure mobilising stemcells (21%). In 2 of those 5 patients harvesting succeeded eventually after additional stimulation with filgrastim and another 2 patients were mobilised in a later stage after an additional course of chemotherapy using filgrastim in high dosage. None of the filgrastim mobilisation procedures failed. A median of 7,2x10^6/kg CD34 cells was obtained, 11x10^6/kg, in fewer procedures. The collected number of CD34 cells per kg bodyweight per ml of processed volume during the apheresis procedure was higher in the filgrastim group, 382x10^6/kg ml (pegfilgrastim) vs. 803x10^6/kg ml (filgrastim). Conclusions. A fixed dose of pegfilgrastim (6 mg) is not sufficient to achieve adequate stemcell mobilisation in all patients. Failure was observed in 21% of the patients. The number of CD34 cells collected and the efficiency of the apheresis procedure appear to be higher in the patient group treated with filgrastim.
CIRCULATING VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) AND ITS SOLUBLE RECEPTORS VEGFR-1 AND VEGFR-2 IN PATIENTS WITH LYMPHOID MALIGNANCY

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Backgrounds. Vascular endothelial growth factor (VEGF) is the most important proangiogenic factor involved in normal and pathologic angiogenesis. Biologic functions of VEGF are mediated by the activation of structurally homologous tyrosine kinase receptors: VEGFR-1, VEGFR-2 and VEGFR-3. The exact role of VEGF receptors in the pathogenesis of lymphoma remains unknown. Aims. The aim of the study was to compare the concentrations of VEGF, VEGFR-1 and VEGFR-2 in the serum of 80 never-treated Non-Hodgkin’s lymphoma patients in different stages of the disease (35 of these patients were diagnosed with the aggressive lymphoma and the remaining 45 patients with the indolent lymphoma; the control group consisted of 17 patients with persistent chronic lymph node enlargement). Methods. Serum VEGF, sVEGFR-1 and sVEGFR-2 levels were determined by means of the enzyme-linked immunosorbent assays (ELISA) (R&D Systems, USA) according to the manufacturer’s protocol. Results. The VEGF serum concentration was found to be significantly higher in the aggressive lymphoma group when compared to the control group (median=435 pg/mL and 231 pg/mL, respectively; p=0.02026). In the indolent lymphoma group the VEGF concentration was also higher than in the control group, showing a tendency towards statistic significance (p=0.05792). There was no significant difference as far as VEGF between the two studied lymphoma subgroups. The serum concentrations of soluble VEGFR-1 were significantly higher in patients with both forms of lymphoma when compared to the control group (median=86 pg/mL and 44 pg/mL respectively; p=0.005501). The serum concentrations of the soluble form of VEGFR-2 were significantly higher in patients with the aggressive lymphoma when compared to the indolent lymphoma patients (median=10655 pg/mL and 8985 pg/mL, respectively; p=0.005501). We have not found a correlation between the serum level of VEGF and the soluble forms of its receptors VEGFR-1 and VEGFR-2 in any of the lymphoma patients. We have also checked the ratio as far as the amounts of VEGF and its soluble receptor (activity index VEGF/s-VEGFR-1). Conclusions. The results obtained for VEGF in patients diagnosed with the non-Hodgkin form of lymphoma confirm the role this protein plays in the pathogenesis of lymphoma, especially of its more aggressive form. The higher concentration of VEGFR-2 in the aggressive lymphoma patients when compared to the indolent lymphoma patients shows that - apart from the VEGF concentration - also the concentration of its receptors (especially its second receptor) has an influence on the course of lymphoma. This shows that not only VEGF but also its receptors should be the aim of the antiangiogenic therapy. To sum up, concentrations of VEGF and its VEGFR-2 may have an important influence on the course of non-Hodgkin’s lymphoma.

INCREASED MT1-MMP EXPRESSION IS INVOLVED IN G-CSF-INDUCED MOBILIZATION OF HUMAN CD34+ HEMATOPOIETIC PROGENITOR CELLS

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Backgrounds. G-CSF is the most established agent for hematopoietic progenitor cell mobilization in clinical practice. G-CSF-induced mobilization is initiated by activation of neutrophils, which secrete various matrix metalloproteinases (MMPs) and serine proteases. These soluble enzymes degrade bone marrow (BM) extracellular matrix (ECM) and modulate cytokines and receptors, leading to a disruption of cell-cell and cell-matrix interactions and, ultimately, release of progenitors. Yet, progenitor mobilization by G-CSF was apparently normal in mice lacking these soluble enzymes. Therefore, we hypothesized that membrane type 1-matrix metalloproteinase (MT1-MMP), or membrane-bound MMP, might be also required for progenitor cell egress. MT1-MMP is a key enzyme for normal cell motility and tumor cell migration and invasion. Methods and Results. We found that human CD34+ cells express variable surface MT1-MMP levels, depending on the cell source and G-CSF treatment. The highest expression was found on CD34+ cells enriched from BM and mobilized peripheral blood (MPB) of G-CSF-treated donors (mean fluorescence intensity >900 and 159±40, respectively). MT1-MMP expression in vitro increased two-fold membranial MT1-MMP expression as compared to IL-6- or SCF-stimulated or untreated human CD34+ BM cells from healthy donors (steady state BM). Importantly, in vivo progenitor mobilization by five daily injections of G-CSF was accompanied by increased MT1-MMP mRNA and protein levels in both bone BM mononuclear cells and human hematopoietic mature and progenitor cells in pre-clinical model of NOD/SCID mice engrafted with human hematopoietic cells. Immunocytochemical analysis of human CD34+ cells plated on hyaluronate-coated cover slips revealed that in response to SDF-1, MT1-MMP changes its localization in the polarized and spreading cells, suggesting a role in the process of progenitor directional migration. Indeed, blocking MT1-MMP function by antibody (Ab) or its endogenous inhibitor-TIMP-2 slightly but significantly reduced the in vitro chemotactic response of human MPB-derived CD34+ cells through uncoated transwells filters. The effect of MT1-MMP neutralization was even more prominent (60% inhibition) on the CD34+ cell chemotaxis via Matrigel, i.e., ECM barrier. Importantly, in vivo administration of human specific function blocking MT1-MMP Ab in the course of G-CSF treatment of NOD/SCID chimeric mice almost completely abrogated G-CSF mobilization of human maturing CD45+ leukocytes, immature CD34+ cells and the more primitive CD34+/CD38-low progenitor cells. Finally, analysis of samples obtained from peripheral blood of 29 patients with lymphoid malignancies treated with chemotherapy and G-CSF revealed bone marrow (BM) extracellular matrix (ECM) degradation and an increased number of mononuclear cells and CD34+ progenitors on the day of first apheresis. Conclusions. We suggest that following G-CSF treatment, increased levels of MT1-MMP on the surface of human progenitors in the BM facilitates their mobilization most probably due to pericellular ECM degradation and/or ECM signaling. Our data indicate that MT1-MMP plays an essential role in clinical mobilization procedures, and might serve as a target molecule for new approaches to enhance the mobilization efficiency.

ROLE OF SONIC HEDGEHOG FOR REGULATING THE PROLIFERATION, MIGRATION AND DIFFERENTIATION OF HEMANGIOBLAST IN THE MICROENVIRONMENT OF AGM REGION

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Background. Recently, it was reported that the intra-embryonic aorta-gonad-mesonephros (AGM) region exclusively and autonomously generated hemangioblasts for hematopoietic and endothelial system and dramatically increased hemangioblasts numbers thereafter. It is logically believed that the microenvironment of this region implicated in the
There was an increased level of phospho-PI3-kinase in mediated proliferation and migration in BM-EPCs. The results also suggest that the angiogenic effects of shh involve in the PI3-kinase pathway.

Results. The proliferation of BM-EPCs was promoted when co-cultured with AGM-derived stromal cells, and the effects could be blocked by antibody of Shh. The proliferation of hemangioblasts were strengthened further and Shh-N-Terminus was added into the cultural supernatant of hemangioblasts. Shh was observed to be induced to apoptosis or differentiation after a short time of proliferation. Furthermore, the ability of migration could be promoted in the co-cultured hemangioblasts by adding exogenous Shh-N-Terminus. Conclusion. Shh pathway could have an effect on the proliferation, differentiation, apoptosis and migration of hemangioblasts, but the roles were regulated by the microenvironment surrounding the cells.

**0864 SONIC HEDGEHOG PROTEIN PROMOTE BONE MARROW-DERIVED ENDOTHELIAL PROGENITOR CELL PROLIFERATION, MIGRATION AND VEGF PRODUCTION VIA PHOSPHATIDYLINOSITOL 3-KINASE/AKT SIGNALING PATHWAYS**

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**Backgrounds.** It was reported that shh pathway was involved in de novo vascularization of certain embryonic tissues as well as in inducing angiogenesis in an adult mammalian system. But if shh signal pathway plays a role in regulating EPCs behavior has not clear yet. Hence, the purpose of the present study was to address the question of whether shh protein effects BM-EPCs proliferation and migration, and then to further investigate the signaling mechanisms involved. **Aim.** To investigate the effects of Sonic hedgehog (shh) protein on bone marrow-derived endothelial progenitor cells (BM-EPCs) proliferation, migration and vascular endothelial growth factor (VEGF) production, and the potential signaling pathways of these effects involved. **Methods.** Bone marrow-derived CD133+ cells were enriched using the MACS system from adult bone marrow and then BM-EPCs were cultured in gelatin-coated culture dishes. The effects of shh N-terminal peptide on BM-EPCs proliferation were evaluated using the MTT colorimetric assay. Cell migration was assayed using a modified boyden chamber technique. The production of VEGF was determined by enzyme-linked immunosorbent assay (ELISA) and immunofluorescence staining. The potential involvement of PKC and PI3K signaling pathways was explored using selective inhibitor or western blot. **Results.** The proliferation, migration and VEGF production in BM-EPCs could be promoted by exogenous shh N-terminal peptide at concentrations from 0.1 µg/mL to 10 µg/mL, and could be inhibited by anti-shh antibodies. Shh-mediated proliferation and migration in BM-EPCs could be partly attenuated by anti-VEGF. There existed a significant increase in phospho-PI3-kinase in newly separated BM-EPCs, and the expression of phospho-PI3 kinase increased significantly when exogenous shh N-terminal peptide added, but could be attenuated by anti-human/mouse shh-N-terminal peptide antibody. Moreover, the inhibitor of the PI3-kinase, but not the inhibitor of the PKC significantly inhibited the shh-mediated proliferation, migration and VEGF production. Conclusion. Shh protein can stimulate bone marrow-derived BM-EPCs proliferation, migration and VEGF production, which may promote neovascularization to ischemic tissues. **Discussion.** There was an increased level of phospho-PI3-kinase in mediated proliferation and migration in BM-EPCs. The results also suggest that the angiogenic effects of shh involve in the PI3-kinase/Akt signaling pathways.
thyroid maturation (days 3-9), whereas a gradual decrease was observed after day 12. In contrast, TR2 protein was undetectable at day 3, similar to TR-1. TR-2 band intensity peaked on day 9 and then gradually declined (especially after day 12). H- and L-ferritin mRNA levels peaked at day 8. Thereafter, a significant decline was observed throughout thyroid maturation; this decline was more pronounced for L-ferritin. H- and L-ferritin protein bands were first detectable after day 3 and peaked at days 6-9. Starting from day 3 up to terminal differentiation, IRP-1 band intensity increased x4-6 fold; IRP-2 was undetectable. These findings suggest that: (i) TR2 mRNA isoforms are differentially expressed throughout erythropoiesis. (ii) TR1 and TR2 expression during erythroid maturation is controlled by transcriptional and post-transcriptional mechanisms, especially at more advanced differentiation stages; in contrast, H- and L-ferritin expression is mainly regulated at the post-transcriptional level. (iii) The abundance of IRP1 in erythroid cells and its upregulation during erythroid maturation are evidence of its important role in post-transcriptional regulation of both TR1/2 and ferritin.

**0867 THE SPLEEN AS AN EMBRYONIC HEMATOPOIETIC SITE: IDENTIFICATION OF FETAL SPLEEN PROGENITORS**

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**Backgrounds.** During fetal life, the spleen is capable to sustain hematopoiesis. Contrary to the fetal liver and thymus, the fetal spleen (FS) contribution to hematopoiesis remains largely unknown. We have previously shown that FS stromal microenvironment does not sustain the hematopoietic stem cells (HSC) proliferation and multipotency. In contact with the FS stroma, HSC differentiate toward the myeloid lineage while their lymphoid engagement is prevented. However, the development of B cells is sustained in the FS when hematopoietic progenitor already possesses a lymphoid signature. **Aims.** To understand FS hematopoietic capacities, we identified hematopoietic progenitors that are present in the early stages of development (between 14.5 to 15.5 dpc). **Methods.** In the FS, we have isolated a CD4int lineage negative (Lin-) population by cell sorting and analyzed its in vitro and in vivo hematopoietic potentials. By limiting dilution assays and clonal assays, the frequency of B, NK, T and myeloid potentials were assessed. We tested the capacity of injected FS CD4int Lin- cells to reconstitute Rag2/-/- mice. By the use of RAG2-GFP mice, the CD4int Lin- population was further characterized. Moreover, by quantitative RT-PCR, we compared gene expression between FS CD4int Lin- population and other progenitors. **Results.** The FS CD4int Lin- population possesses lymphoid and myeloid potential in vitro. This population keeps its hematopoietic capacities in vivo since CD4int Lin- cells are able to reconstitute the lymphoid and the myeloid compartments of Rag2/-/- mice. The FS CD4int Lin- population could be subdivided into three subsets depending on the level of Rag2 expression (Rag2+, Rag2low and Rag2high). The Rag2- subset is primarily composed of myeloid precursors and we showed that the loss of the myeloid potential is concomitant to the up-regulation of the Rag2 expression. By clonal assays, we displayed that the Rag2lo population is enriched by T/NK progenitors whereas the Rag2hi is mainly restricted toward the B lineage. After 4 days of FS organ cultures, CD4int cells are disappearing while lymphocytes are appearing suggesting that FS lymphocytes derived from in situ differentiation. Moreover, we have determined that the CD4int Lin- population are also the progenitors of the FS CD4hi lymphoid tissue inducer cells that may play a role in the FS architecture. **Conclusion:** The FS CD4int Lin- population encloses several progenitors that are engaged towards different lineages. These progenitors certainly give rise to committed hematopoietic cells in situ, indicating that the FS actively sustains the lymphoid.

**0868 DIMINISHED PROTEASOMAL DEGRADATION RESULTS IN ACCUMULATION OF GFI1 PROTEIN LEVELS IN MONOCYTES**

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**Backgrounds.** Gfi1 is a transcriptional repressor essential during myeloid differentiation. Gfi1/-/- mice exhibit a block in myeloid differentiation resulting in the accumulation of an immature myelomonocytic cell population and the complete absence of mature neutrophils. Even though mRNA levels of Gfi1 appear to be very low in monocytes, Gfi1 might play a role in the maturation of myelomonocytic lineage as Gfi1/-/- mice exhibit diminished monocyte-derived dendritic cells and disturbed cytokine production by macrophages in response to LPS. **Aims.** Study the role of Gfi1 in monocyte differentiation. **Methods.** Gfi1 mRNA and protein levels were measured by qPCR and Western blot analysis respectively. Modifications of Gfi1 with ubiquitin were analyzed with ubiquitinisation assays using His-tagged ubiquitin and binding of Gfi1 to gene promoters was analyzed with chromatin immuno-precipitation assays. **Results.** Upon forced monocytic differentiation of U937 cells, Gfi1 mRNA levels dropped but protein levels increased indicating that Gfi1 protein expression is mainly regulated post-transcriptionally. To study this we performed ubiquitination experiments and found that Gfi1 is efficiently targeted by the ubiquitin-proteasome pathway. Treatment of cells with proteasome inhibitors MG132 or Velcade resulted in significant increases of both transfected as well as endogenous (U937) Gfi1 levels. Remarkably, after PMA induced monocytic differentiation of U937 cells proteasomal inhibition did not result in an increase in Gfi1 U937 levels. In line with this, we found that radioactive labeled in vitro translated Gfi1 was rapidly degraded in lysates taken from U937 cells, a process which could be blocked by proteasome inhibition. When lysates were taken from PMA stimulated U937 cells, the Gfi1 turnover was significantly delayed. Thus, during PMA forced differentiation of U937 cells Gfi1 protein levels rise due to diminished degradation. Similar findings were found in primary cells. Gfi1 mRNA levels were low in primary monocytes while the protein was clearly detectable. Conversely, Gfi1 mRNA levels were high in granulocytes but the protein was swiftly degraded by the proteasome in these cells. Chromatin immunoprecipitation experiments showed that Gfi1 binds to the promoter of several granulocyte-specific genes in primary monocytes, including C/EBPα, neutrophil elastase and Gfi1 itself. The binding of the repressor Gfi1 to these promoters correlated with low expression of these genes in monocytes compared to granulocytes. **Conclusions.** Gfi1 undergoes efficient ubiquitin-proteasomal degradation in immature hematopoietic cells. Upon monocytic differentiation Gfi1 protein levels increase due to diminished proteasomal degradation, despite low RNA levels. Our data fit a model in which Gfi1 protein levels are induced in primary monocytes to repress genes that play a role in granulocytic differentiation.

**0869 HIGHER CONSTITUTIVE NF-KB SIGNALING IS A DISTINCTIVE FEATURE OF UMBILICAL CORD BLOOD CD34+ PRECURSORS**

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**Aim.** Study the role of GFI1 in monocyte differentiation. **Background.** The binding of the repressor Gfi1 to these promoters correlated with low expression of these genes in monocytes compared to granulocytes. **Conclusions.** Gfi1 undergoes efficient ubiquitin-proteasomal degradation in immature hematopoietic cells. Upon monocytic differentiation Gfi1 protein levels increase due to diminished proteasomal degradation, despite low RNA levels. Our data fit a model in which Gfi1 protein levels are induced in primary monocytes to repress genes that play a role in granulocytic differentiation.
10,000 genes. Although HSPC from BM and UCBM where very similar, a stringent statistical analysis revealed a set of 61 tags (transcripts) differentially expressed, 45 overrepresented in UCBM and 16 in BM. The set of UCBM-overrepresented genes included both subunits (NFkB2 and RELB) of the NF-KappaB transcription factor complex involved in the sustained activation of NFkB transcription targets, trough the non-canonical constitutive pathway. In addition, factors such as interleukin 1 (IL1A and IL1B), lymphocyte function antigen 1 (CD80, CD81 and CD86), and Toll-like receptors; and leukocyte adhesion molecules (CD11a, CD11b, CD11c, CD18, CD2, CD5, CD10, CD16, CD20, CD21, CD23, CD24, CD32, CD40, CD45, CD54, CD58, and CD112) were more expressed in UCBM. The expression of all these genes was also confirmed by real-time PCR. Finally, clustering of the 1,000 top expressed tags of SAGE libraries for 21 normal human tissues revealed a statistically significant overrepresentation of NFkB cis-regulatory elements, including known NFkB transcriptional targets genes (such as CXCL2, CXCL5, ICAM1, IL8, ILB, NFkB2 and RELB), and novel potential targets of NFkB signaling (like RGS1, zyxin and others). NOTCH1, which controls the transcription of NFKB, was also overrepresented in UCBM. Conclusions. Our results point out to a central role of the NF-kB pathway on the molecular and functional differences observed between UCBM and UCBM HSPC. Moreover, NFkB inhibition is known to cause apoptosis and loss of clonogenic function in HSPC. Furthermore, UCBM HSPC rapidly differentiates into T cell on fetal thymic organ cultures, while BM HSPC have to be pre-treated with TNF. Thus, NFkB transcription targets and other UCBM overexpressed genes such as MIP1B, MIP2B, IL1, IL1, RGS1, zyxin, ICAM1, TGFb, LTb, TNF, may be responsible for the differences related to cell survival, quiescence, mobility and adhesion of these cells, as well as increased T cell divergence in the context of this mechanism. It is possible to understand the future studies and to potentially new strategies to stem cell graft manipulation to improve the outcome of transplants with these cells, as well as their handling on propagation cultures.

**0870**
CLOSE FUNCTIONAL SIMILARITIES BETWEEN HUMAN MESENCHYMAL STEM CELLS, PERICYTES AND FIBROBLASTS

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Backgrounds. Mesenchymal stem cells (MSC) are pluripotent precursors capable of differentiating into osteoblasts, adipocytes and chondrocytes, present in bone marrow (BM) and in various other adult and fetal tissues. MSC share many properties with pericytes, which form a continuous structural network of the muscular bed, while the name stromal cell or fibroblast is often used interchangeably with MSC. Aims. To evaluate how similar are MSC obtained from different human tissues and what is their relationship with fibroblasts and pericytes on the basis of their gene expression and functional properties. Methods. A set of 30 genes was selected on the basis of previous results of serial analysis of gene expression (SAGE) for MSC, CD34+ cells, fibroblasts and pericytes. Gene expression of selected genes was measured by real-time PCR or semiquantitative RT-PCR. Results. All MSC and the pericyte culture had the capacity to differentiate in vitro into adipocytes, osteoblasts and chondrocytes, whereas fibroblasts did not differentiate under similar conditions. Cluster analysis of gene expression profiles using Name 4.0 showed that all the MSC lines formed a very close cluster which included pericytes and fibroblasts, separated from normal human cells. In addition, all the MSC lines and pericytes had similar immunophenotypic markers and capacity for in vitro differentiation. Similarity of the gene expression profiles of MSC and fibroblasts were further confirmed by clustering of the 1,000 top expressed tags of SAGE libraries for 21 normal human tissues. Despite the similarity, genes related to angiogenesis, especially CXCL6, were more expressed in MSC from umbilical vein, from adult saphena vein and in pericytes. Differentiation into adipocytes or osteoblasts was accompanied by the increased expression of specific genes, although the global patterns were still very similar, so that they remained in the same cluster together with the MSC. Conclusions. MSC that can be obtained from a variety of adult and fetal tissues and which have very similar immunological markers, differentiation potential and gene expression profiles. Comparison of these characteristics also shows that human MSC, pericytes, and fibroblasts are very closely related, representing probably different functional states of the same cell. Identity between MSC and pericytes is particularly striking, whereas fibroblasts seem to have lost most of its differentiating potential. These results have practical as well as conceptual applications, since they demonstrate the functional equivalence of pericytes and MSC from different origins, and their close relationship to fibroblasts.

**0871**
IDENTIFICATION OF NOVEL REGULATORS OF HEMATOPOIETIC STEM CELL MOBILIZATION

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In an effort to identify the molecular changes during hematopoietic stem cell (HSC) regeneration we have analyzed genome wide changes in gene expression in HSCs in response to the chemotherapeutic agent cyclophosphamide (Cy) and the growth factor granulocyte colony stimulating factor (G). Cy/G treatment leads to an initial loss of proliferative precursors, followed by a rapid expansion of stem and progenitor cells and their subsequent migration to the peripheral blood. While this approach has been capitalized on for harvesting hematopoietic stem cells in clinical therapy, the regulation of this process remains less well understood. To analyze the molecular changes that occur as HSCs regenerate, we compared gene expression of HSC fractions from untreated mice and mice treated with Cy or G. Among the genes upregulated by Cy/G, a large majority were a consequence of synergistic activity between Cy and G, while the rest of the upregulated genes could be attributed to G activation by Cy alone. To test whether this screen allowed identification of novel genes that regulate HSC function we analyzed the role of transforming growth factor-beta inducible gene-h3 (big-h3), a gene highly upregulated after Cy/G treatment. big-h3 is an extracellular matrix protein that mediates the adhesion and migration of various cell types; however its role in HSCs is unknown. Our experiments indicate that overexpression of big-h3 in HSCs lead to accelerated differentiation in vitro. Consistent with this, transplantation of big-h3-overexpressing HSCs resulted in reduced chimerism in vivo, and ultimate exhaustion of the HSC compartment. These experiments suggest that an enhanced ability to differentiate may be an important element of hematopoietic regeneration after injury, and that big-h3 is a regulator of such processes. These data also indicate that further functional analysis of other candidates identified through this comparative gene expression analysis will likely lead to identification of other novel regulators of HSC development and mobilization.
Background. Acute myeloid leukaemia (AML) is a haematopoietic stem cell (HSC) disease. Although chemotherapy is initially successful in the majority of AML patients, many patients relapse, suggesting that therapies are ineffective in eliminating the leukemia stem cells (LSCs). Although the AML CD34+CD38- compartment is enriched for LSCs, not all LSCs can be CD34+CD38-; e.g. in CD34 negative AML. An alternative stem compartment is the so-called side population (SP). SP cells are defined by their ability to efficiently efflux Hoechst 33342 dye and in normal bone marrow (nBM) are enriched for HSC activity. In AML, the SP compartment is able to initiate leukaemia in NOD/SCID mice. The exact immunophenotype and the relationship with the CD34+CD38- stem cells are largely unknown. Aim: to define the immunophenotype of AML SP cells in relation to nBM SP cells. Such information may enable AML SP stem cell detection at all stages of disease, to optimise treatment and ultimately guide stem directed therapies. Methods. Using Hoechst dye and antibodies against CD34, CD38, CD7, CD19 and CD56 (the latter three offering leukaemia associated phenotypes, LAP used for MRD detection), as well as a C-type Lectin-like molecule-1 (CLL-1), a marker for the AML CD34+CD38- compartment (van Rhenen et al, Blood 2005; 106: 4), SP immunophenotyping was performed on FACS sorted samples on 106 AML patient samples. Results. The AML patients had a median SP frequency of 0.01% (range 0.003-0.17%). For the immunophenotype, in terms of CD34 and CD38 expression, we found i) the whole blast compartment was partly CD34+ and partly CD34-CD38+ in the 5/6 CD34+ positive (>1% CD34- cases and almost completely CD34-CD38+ in the 3 CD34 negative cases); ii) In 5 CD34+ cases SP cells were in majority CD34+CD38-; The rest was mainly CD34-CD38- ; iii) also in the 3 CD34- cases CD34-CD38+ was the predominant phenotype (located in the very small CD34- compartment); iv) in all cases there was only a very small CD34+CD38 compartment (median 4% of SP cells). LAPs present on the whole blasts were also present on the SP cells in all 6 LAP+ cases, indicating malignancy. SP cells from nBM samples were completely LAP negative. FISH analysis in a (t(8;21)) AML patient confirmed SP malignancy. In all 8 cases SP cells were partly or completely positive for CLL-1. SP cells from nBM were completely CLL-1 negative. Summary/Conclusions. Our results suggest that the phenotype of AML SP cells not necessarily reflects that of the whole blast compartment and in addition to being reported as CD34+CD38- or CD34-CD38+, in most cases is CD34+CD38-: CLL-1 and LAP expression on AML SP cells, similar to CD34+CD38- stem cells, offers the ability for stem cell detection under MRD conditions and especially in cases of CD34+CD38- AML. Using the SP as a CD34- stem cell compartment. In addition, CLL-1 expression on both AML SP and CD34-CD38- LSCs and on normal HSCs offers putative potential of toxic coupled antibody stem cell therapies now covering all known AML stem cell phenotypes.

0875 HUMAN UMBILICAL CORD BLOOD CELLS REGENERATE HEPATOCYTES IN A NON-MYEOABLATIVE SETTING
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Backgrounds. A number of reports have shown that rodent bone marrow cells can transdifferentiate into hepatocytes. Human umbilical cord blood (HUCB) is a rich source of haematopoietic stem cells and mesenchymal progenitor cells which might be used for tissue or organ repair. Aims. We evaluated whether human umbilical cord blood cells infused following non-myeloablative conditioning can regenerate hepatocytes after acute liver injury in an immuno-competent mouse model. Methods. In an acute hepatic injury model, female C57Bl6 mice were administered toxic dose of acetaminophen. Six hours later, the mice were given Sudarabine (0.5 mg/kg) and cyclosporine (5 mg/kg) followed by infusion of human umbilical cord blood mononuclear cells at a dose of 1x10^7 mononuclear cells per kilogram of body weight. The cyclosporine was
continued at 3mg/kg daily for four more days. Surviving mice were sacrificed at two and four weeks post transplant. Fluorescence in-situ hybridization (FISH) and polymerase chain reaction (PCR) analysis of hepatic DNA for Χ-satellite region of human chromosome 17 were used to confirm the presence of hepatocytes from human origin. Results. Fifteen out of 24 mice received umbilical cord blood cells infusion after non-myeloablative conditioning survived beyond two weeks compared with two of the 11 control mice (p= 0.027). The surviving mice were sacrificed at two weeks and four weeks post-transplants. Histological sections showed regenerating liver with hepatocytes of normal appearance comparing with the livers of the control mice showing extensive necrosis. FISH analysis confirmed the presence of human X-chromosome positive cells in the hepatic sections of all but three of the surviving mice, the percentages ranged from 0.5-9%. PCR analysis showed that all except 3 mice (lanes 9, 12 and 13) showed presence of about 1.5-20% human DNA (Figure). In three mice (lanes 6, 14 and 15), about 10-20% of the hepatic DNA was of human origin. There was a concordance between the proportion of human DNA detected by PCR and the percentage of human Y-chromosome positive cells by FISH. Non-hepatic tissue (heart and kidney) did not contain human DNA. Conclusion: Our data suggested that human umbilical cord blood could repair acetamino-phen induced acute hepatic injury in a non-myeloablative setting. Our model closely mimics the clinical UCBB transplantation setting and should be further explored in a clinical setting. In the future, this may be an effective approach in the management of patient with fulminant hepatic failure waiting for orthotopic liver transplantation.

### 0877

**INTRACORONARY AUTOLGOGOUS BONE-MARROW STEM CELL TRANSFER AFTER MYOCARDIAL INFARCTION: PRELIMINARY RESULTS OF A RANDOMISED TRIAL**


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**Backgrounds.** Recent experimental and clinical studies have shown that cardiac transfer of stem cells and progenitor cells derived from bone marrow may enhance functional recovery after acute myocardial infarction (AMI). Aims. To assess whether intracoronary transfer of autologous bone-marrow cells could improve left ventricular remodelling and global ejection fraction after 6 months' follow-up. Methods. A total of 40 patients with AMI, were randomly assigned to either a control group (n=20) that received optimum postinfarction medical treatment, or a bone-marrow-cell group (n=20) that received optimum postinfarction medical treatment and intracoronary transfer of autologous bone-marrow cells in the first week after the primary percutaneous coronary intervention. Autologous bone marrow stem cells were obtained by iliac crest aspiration. After density gradient centrifugation, mononuclear cells were incubated for 24h in Teflon bags with X-vivo medium at 37°C. Prior to intracoronary infusion, cells were washed and reseeded in 10 ml normal saline. We assessed left ventricular volumes and function from baseline to a minimum of 6 months' follow-up by cardiac magnetic resonance imaging. Results. Median volume of bone marrow aspirate was 36 ml (range 30-40), with a total number of mononuclear cells of 137 million (65-400). The number of infused cells was 80 million (20-215), with a viability of 90 ±12%, and a recovery rate of 59 ±19%. The content of CFU-GM and BFU-E was 45 ±21 and 150 ±106 per dish, respectively. No infusion related complications were observed. Global left ventricular ejection fraction (LVEF) at baseline was 43.7% ±14.2 in controls and 49.3% (p=0.64) in the bone-marrow-cell group. No significant differences in ventricular volumes were found between both treatment groups. Conclusions. In patients with marked left ventricular dysfunction after AMI, we haven't found significant improvement of LVEF in the bone-marrow-stem-cell group after 6 months' follow-up. The potential impact on long-term survival needs further evaluation.

### 0876

**TOWARD STANDARDIZATION OF CELLULAR PRODUCTS FOR IMMUNOMODULATION AND REGENERATIVE MEDICINE. EXPANSION OF MESCENHYMAL STEM CELLS DERIVED FROM AMNIOTIC FLUID: PERSPECTIVES OF FUTURE CLINICAL APPLICATION**

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Background. Mesenchymal stem cells are now extensively studied in projects involving either their immunomodulatory property and their utilization in regenerative medicine. Because of the limited absolute number of bone marrow (BM) derived MSC available as cell therapy product we addressed our attention to MSC derived from alternative sources. Recently, it has been suggested that amniotic fluid (AF) is a rich source of MSC. Aim. In the current study, we evaluated the isolation and expansion of AF-MSC testing the immunophenotype and karyotype stability at different passages. The expansion of MSC derived from BM and AF were compared. Methods. Cell isolation from AF and BM. Second trimester samples of AF were centrifuged for 10 minutes at 400g. Cells were plated in Mesencult medium and, after 48 hours, non adherent cells were discarded. The expansion of AF-MSC was assessed plotting the density of the cultures, the percentage of cell positive for CD45, CD34, CD133, CD90, CD105, and CD166, and the karyotypic stability at different passages. Results. AF-MSC showed higher expansion capability when comparing with the livers of mice survived.
LOCAL INJECTION OF BONE MARROW CELLS AUGMENTS THE NEOVASCULARIZATION IN A MOUSE ISCHEMIC HIND LIMB BY INDUCING VEGF

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Backgrounds. Improved neovascularization is an important therapeutic goal after myocardial infarction and limb ischemia. In the recent years, increasing evidence suggests that bone-marrow derived circulating cells home to the sites of ischemia and contribute to the formation of new blood vessels, so the direct injection of bone marrow cells (BM-MNCs) in the ischemic sites might augment angiogenesis and collateral vessel formation. Aims. We examined whether the BM-MNCs might induce the angiogenesis as effectively as endothelial progenitor cells (EPCs) in a mouse model of hind limb ischemia and evaluated the expression of related molecules. Methods. BMCs and EPCs were obtained from C57BL/6. Unilateral hind limb ischemia was surgically induced by femoral artery ligation in C57BL/6 mice (control group; n=4), autologous BM-MNCs (Group 1; n=4), and autologous BMCs and EPCs (Group 2; n=4, 1.1x0.21 x10^7/animal) were transplanted into the ischemic limbs after 10 days. After 4, 8, 12 weeks, the capillary/muscle ratios were evaluated. And VEGF, eNOS, ProMMP-9 and MMP-9 were assayed in tissue homogenates using western blot analysis, too. Results. Injected PKH2 labeled BM-MNCs were observed for 12 weeks after transplantation the group 1, group 2 had a higher capillary/muscle ratio (1.27±0.08 vs 0.82±0.12) than control (0.62±0.08). The expression of MMP-9 was normalized within 14 days after transplantation. Figure 1. The expression of VEGF by IHC.

ABNORMALITIES OF BONE MARROW MESENCHYMAL STEM CELLS IN PATIENTS WITH CHRONIC IDIOPATHIC NEUTROPENIA

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Backgrounds. Chronic idiopathic neutropenia (CIN) is an acquired underproduction neutropenia syndrome characterized by hypoplastic and left-shifted granulocytic series in the bone marrow (BM). Previous studies have shown that the bone marrow (BM) microenvironment may contribute to the pathophysiology of the disease by producing pro-inflammatory cytokines and chemokines and providing a defective support of granulocytosis. Whether, however, there is a primary defect at the mesenchymal stem cell (MSC) level in these patients remains unknown. Aims. To study the reserves, the functional characteristics and the differentiation potential of BM MSCs in patients with CIN. Methods. Thirteen patients with CIN and 22 age- and sex-matched healthy controls were studied after informed consent. All patients had neutrophil counts below 1800/microliter and were satisfying the previously reported diagnostic criteria for the disease. The BM mononuclear cells (BM-MNCs) were isolated from posterior iliac crest aspirates and the MSCs were expanded according to a standard protocol. MSCs were characterized by their immunophenotypic characteristics (CD45+ CD44+, CD73+ CD90+, CD105+, CD146+) and their adipogenic (Oil red O stain and aP2 and PPAR-γ expression by RT-PCR), osteogenic (ALP/Von Kossa stain and ALP and CBFA1 expression by RT-PCR), and chondrogenic (Masson and Alcian blue stain and Collagen II and Aggrecan expression by RT-PCR) potential after induction of differentiation in appropriate media. The frequency of MSCs in the BMNC fraction was evaluated by means of a limiting-dilution assay (LDA) based on the Poisson probability. The functional characteristics of MSCs were studied by evaluating (a) their clonogenic potential using a standard colony forming unit-fibroblast (CFU-F) assay and enumerating the CFU-Fs/10^6 BMMCs plated through passages (P), (b) their proliferative potential-time course by using the MTT assay and evaluating the cell doubling time (2ⁿ=cells counted/cells plated) in each passage. Results. CIN patients displayed normal number 14.64±14.53 MSCs/10^5 BMMCs in the patients versus 23.78±6.49 MSCs/10^5 BMMCs in the controls; p=0.1986) and normal immunophenotypic characteristics of BM MSCs. The chondrogenic, osteogenic and adipogenic potential of patient MSCs did not differ from the respective of the controls as was evaluated by the collagen II and aggrecan, the ALP and CBFA1, and the aP2 and PPAR-γ mRNA expression, respectively, by means of a semi-quantitative RT-PCR. Compared to healthy controls, however, patient MSCs displayed impaired CFU-F potential time-course (p<0.001; P1-P6) as well as impaired proliferative capacity. This was demonstrated by the MTT assay (p<0.01 at P1) and the cell doubling time-time course (p<0.001; P1-P7). Summary/Conclusions. Patients with CIN display normal number and differentiation potential of BM MSCs. The clonogenic and proliferative potential of patient MSCs, however, is defective as compared to the respective of the healthy controls. Analysis of the production of growth factors and inhibitors at protein level as well as the telomeric length of patient MSCs is currently under investigation to elucidate further the pathophysiologic basis of the observed MSC abnormalities in CIN patients.

CONGENITAL NEUTROPIA: A GROUP OF DISORDERS WITH GENETIC AND PHENOTYPIC HETEROGENEITY

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Severe congenital neutropenia (CN) is a general term for a group of disorders characterized by extremely low blood neutrophil counts (ANC < 0.5x10^9), early stage maturation arrest of myelopoiesis, and recurrent hematologica/the hematology journal | 2006; 91(s1) | 323
bacterial infections. In general more than 90% of CN patients respond to daily G-CSF treatment with a sustained neutrophil increase resulting in a significant reduction of infections and an improved quality of life. However, besides neutropenia, the presence of various concomitant clinical features in subpopulations of patients in conjunction with an increased risk of leukemic transformation in about 10% of all CN patients strongly suggested to search for new sub diagnoses to identify patients at risk for leukemia. The Severe Chronic Neutropenia International Registry (SCNIR) has collected longitudinal data on more than 400 patients with various causes of CN. This unique resource of data was used to classify different subtypes of CN, to estimate the relative frequency of these conditions and to correlate them to leukemic transformation. Our classification scheme is as follows: 1. By inheritance: autosomal dominant, autosomal recessive, sporadic CN. 2. By genetic aberrations - ELA2, GFI-1, WASP, P14 related CN, SBDS related CN (Shwachman-Diamond syndrome), G-CSF responsive or nonresponsive CN, with or without acquisition of G-CSF-receptor mutations, with or without an evolution of osteopetrosis/osteoporosis, or the presence of concomitant dysplastic features (e.g. organ abnormalities). Recent research and the work of the SCNIR lead to a significantly improved classification of CN. The identification of subtypes of CN, their distinctive risks of malignant transformation, and their responses to treatment has contributed substantially to our general understanding of the problem of neutropenia. This knowledge now also allows clinicians to provide patients and families with much more accurate prognostic information and better guidelines for therapy.

SUPERIOR EFFECTS OF HIGH DOSE ENZYME REPLACEMENT THERAPY IN TYPE 1 GAUCHER DISEASE ON BONE MARROW INVOLVEMENT AND CHITROTOSIDASE LEVELS; A TWO CENTER RETROSPECTIVE ANALYSIS

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Background. Gaucher disease type I is the most common lysosomal storage disorder, caused by deficient activity of the enzyme glucocerebrosidase (OMIM *606465), leading to the accumulation of glucocerebroside in spleen, liver and bone marrow. The most important clinical manifestations are hepatosplenomegaly, cytopenia and skeletal involvement. Gaucher disease can be treated with enzyme replacement therapy (ERT), leading to a dramatic clinical response in most patients. Even after more than 14 years of experience, the most effective dosing regimen of ERT is still a subject of debate and varies from 15-130 U/kg/month, making a huge economic difference of 55.000 up to 112.000 per year. Aim. The aim of the study was to retrospectively compare long term outcome on hematological, visceral and biochemical parameters in two different dosing schedules (15-30 U/kg/4 weeks vs 80 U/kg/4 weeks). Methods. Adult Gaucher disease type I patients from two large European treatment centers, Amsterdam (AMC, N=49, median dose 15-30 U/kg/4 weeks) and Düsseldorf (HHU, N=57, median dose 80 U/kg/4 weeks) were included. Follow-up parameters included hemoglobin, platelet count, plasma chitrotosidase levels, liver and spleen dimensions, severe bone complications and scoring of bone marrow involvement by MRJ of the femo-
ra. All parameters were matched at baseline and analyzed in two separate ways: comparison of baseline values vs values after one year and life table analysis (Kaplan Meier). Results. There were no significant differences in genetic background, age, gender, number of splenectomies and SSI in any of the matched populations. Improvement in hemoglob-
in, platelet count and hepatosplenomegaly was not significantly different between both cohorts, whereas bone marrow involvement by MRJ especially in patients with severe bone disease, and plasma chitrotsi-
dase improved significantly faster in the higher-dosed group. Major bone complications rarely occurred in both groups. Conclusions. As improvement of hemoglobin, platelet count and liver and spleen volume is not dose-dependent, extensive organomegaly and cytopena do not justify a high initial dose. The quicker response for bone marrow involvement upon a higher dose in severely affected patients is considered an important criterion to start a higher dose of enzyme. Chitrotsidase proves to be a sensitive indicator of dose effects and may be used in that respect to monitor response. The determination of the most cost-effective dosing regimen should be made individually and on the basis of a complete disease profile, including proper assessment of bone marrow involve-
ment in addition to hematological, visceral and biochemical param-
eters.

LUNG RESECTION FOR INVASIVE PULMONARY ASPERGILLOSIS IN NEUTROPENIC PATIENTS WITH HEMATOLOGIC MALIGNANCIES: LONG TERM RESULTS IN THIRTY CASES


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Invasive pulmonary aspergillosis (IPA) is a major cause of morbidity and mortality in neutropenic patients. Nevertheless, recent studies suggest that the outcome of IPA is improving due to early diagnosis (CT scan, antigenemia), use of new antifungal agents (Azoles and Echinocan-
gises), and possibly IPA recurrence and early surgical resection. We here report a retrospective one center study of 30 cases of IPA treated by sur-
gical treatment from 1998 to 2005. Patients were 18 men and 12 women, with a median age of 30 years (15 - 74). The underlying diseases were AML, ALL, aggressive lymphoma and myeloma in 22, 5, 2 and 1 cases, respectively. Surgery was planned after hematologic recovery from the last course of chemotherapy during which IPA was diagnosed, either possible in 15 cases, probable in 14 cases or proven in 1 case (Ascioglu, CID 2002). Surgery consisted in 1 pneumectomy, 4 bilobectomies, 17 lobectomies, 6 wedge resections and 2 lobectomies with wedge resec-
tions. No perioperative deaths occurred and the median duration of hospitalisation was 12 days. Four patients presented post surgical complica-
tions (pneumothorax, pneumopathy, section of phrenical nerve and bleeding). The diagnosis of definite IPA was confirmed in all 30 cases. Immediately after surgery, 24 patients were able to receive subsequent intensive chemotherapy courses, including 11 stem cell transplant (SCT), either auto (4) or allogenic (7). In all cases, patients subsequently received parenteral antifungal therapy. During these new intensive chemothera-
py courses, recurrent aspergillosis was observed in only 2 cases, (induc-
ing 1 death from brain localisation). Overall, with a median follow-up of 8.8 years (1-18), 86% of the patients are alive and the main cause of mortality was relapse, but not IPA. In conclusion, early surgical resec-
tion together with antifungal therapy allows definite diagnosis of IPA, and in this work, we first analyzed the expression of Notch signal-
ning components in EPCs, isolated from mouse BM and in vessels that grow into xenografted human lymphoma. Results. Mouse EPCs expressed the receptor Notch1 and the ligands Delta-like 4, Delta-like 1 and Jagged 1. In addition, these cells also expressed the Notch down-
stream target gene Hey1, which was upregulated during EPCs differen-
tiation in a significant reduction of infections and an improved quality of life.
lymphoma xenograft. To evaluate in particular the role of Notch ligand DLL4 in EPCs function, we investigated the *in vitro* differentiation potential, cell migration and vessel network formation of EPCs isolated from DLL4+ mutant mice. Conclusions. These results obtained thus suggest that the Notch signalling pathway might play a role in EPCs function, mediating the crosstalk between EPCs and endothelial cells during tumour angiogenesis.

**0884 PRESENT TRENDS IN THE MANAGEMENT OF INVASIVE FUNGAL INFECTIONS IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES**

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**Background.** Fungal infections are an important cause of morbidity and mortality in patients with hematological malignancies. Historically, treatment has been with amphotericin B and later its lipid formulations. However, new therapeutic agents have recently been introduced. The empirical use of antifungal therapy is today a standard approach in patients with persistent febrile neutropenia. However, the low incidence of invasive fungal infections (IFI) and the progress in the diagnoses and treatment of IFI have made the routine use of empirical antifungal therapy questionable. Aims. With the aim to report the present trend in the use of antifungal agents for the treatment of IFI we prospectively observed type, safety and efficacy of given antifungal treatment in patients with hematological malignancies during a recent 18-month-period. At the same time we analyzed the impact of restricted use of empirical antifungal therapy on IFI related mortality. Patient and Methods. Data was collected from patients admitted for treatment of febrile neutropenia to our department from November 2003 through April 2005. All patients who had received antifungal therapy empirically or for treatment of IFI were included. Local guidelines recommended a restricted use of empirical antifungal therapy to patients with persistent febrile neutropenia (5 days or more), who were, after individual assessment, considered to be at high-risk for IFI. Caspofungin was recommended for this indication. Voriconazole was recommended as primary therapy of invasive aspergillosis, while caspofungin in this setting was recommended as salvage therapy. Results. A total of 279 episodes of neutropenia and fever following chemotherapy were recorded. All patients were treated for hematological malignancies, predominantly acute leukemia (50%). Treatment of IFI was given during the management of 41 (14%) episodes of febrile neutropenia occurring in 35 patients (Table). Voriconazole (27 episodes) and caspofungin (14 episodes) were the only antifungal agents used as initial therapy. Two patients received the combination of caspofungin and voriconazole as salvage therapy. The rate of antifungal therapy success rate was 78% (Table). Oral preparation of voriconazole was given from the first day of treatment to 88% of patients treated with this agent. In general, antifungal agents were well tolerated and only two patients had to discontinue treatment due to severe adverse event. The overall 4-week mortality rate was 8%. Two patients died from invasive pulmonary aspergillosis. Empirical antifungal therapy was given to 13 patients with persistent febrile neutropenia without any signs of focal infection and resulted in successful outcome in 92% of cases. In 127 episodes of persistent febrile neutropenia antifungal therapy was deemed unnecessary and accordingly was not administered. In this subgroup of patients the overall 4-week mortality rate was 4% and 4 patients died of infection. No IFI related mortality occurred in this subgroup of patients. Conclusions. A better tolerability and efficacy of voriconazole and caspofungin together with the oral alternative of voriconazole have led to a shift in the use of antifungal agents for the treatment of IFI. A restricted use of empirical antifungal therapy was, in this setting, not associated with increased IFI related mortality.

**Table 1.**

<table>
<thead>
<tr>
<th>Number of episodes</th>
<th>Successful outcome (%)</th>
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<tr>
<td><strong>Empirical therapy</strong></td>
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<tr>
<td>13</td>
<td>12 (92)</td>
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<tr>
<td>Possible IFI</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>18 (78)</td>
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<tr>
<td>Probable IFI</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1 (25)</td>
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<tr>
<td>Proven IFI</td>
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<td>1</td>
<td>1 (100)</td>
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<td></td>
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<td>41</td>
<td>32 (78)</td>
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**0885 A SURVEY ON INVASIVE FUNGAL INFECTIONS IN SCT: TREATMENT, INCIDENCE AND CLINICAL OUTCOME AMONG 686 PATIENTS TRANSPLANTED DURING 2000-2004**


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**Backgrounds.** Historical incidence (80s and 90s) of IFI in recipients of SCT ranges from 10-25% with an overall case fatality rate of up to 70-90%. Aims. Here we report our findings regarding the demographics, microbiology, clinical outcome and risk factors for the development of IFI among patients who underwent SCT in 5 hospitals of Madrid (Spain). Methodology: A retrospective study of all the patients who underwent SCT in 5 units of Madrid (Spain) during 2000-2004 was done. Results: 120 patients received alloSCT (18%), 86 from a sibling donor and 34 from alternative donors. In 59 cases (49%) a RIC regimen was employed. PB was the source of stem cells in 650 patients (98%). Lymphoma (27%), acute leukemia (182) and myeloma (160) were the main underlying diseases. 375 patients were in complete remission and 235 (45%) were transplanted in persistent disease (4%). IFI (91%) was the main cause of death. Conclusions. Determination of serum galactomannan was introduced in the last two years and data were available from 127 cases (20%). Nearly all patients received antifungal prophylaxis (639) oral fluconazole in 576 cases (90%). An empiric antifungal treatment was instaurated in 190 cases (28%) and was more common in the allo population (40% vs 25%, p = 0.007). Ambisome was the drug of choice in 120 cases (62%). IFI after SCT (EORTC criteria) occurred in 32 patients (4.8%), possible: 17, probable: 7, proven: 8. Median day of diagnosis was day +277 (375), and pulmonary disease was the most common clinical presentation. Aspergillus was the most frequently involved mold (60%). 16/32 patients with a diagnosis of IFI had died (80%), in 4/16 cases death was attributed directly to IFI and contributed in other 4 cases. IFI was more frequent among allo vs auto (14.4% vs 2.7%, p < 0.001); AL vs other disease (6.7% vs 5.7%, p = 0.04); a previous history of IFI (17% vs 4%, p < 0.02); severe GVHD (grades III-IV and extense C GVHD) on IS treatment (80% vs 4%, p = 0.0002) and disease not in CR (6.67 vs 3.47, p = 0.059). A multivariate analysis selected type of transplant (allo, p = 0.003; RR: 5.76), previous IFI (p = 0.04; RR: 3.9), and severe GVHD (p = 0.04; RR: 4.02) as the main risk factors for the development of IFI. Conclusions. Our findings shows that IFI had a low impact on mortality in the present series, 9/660 (1.3%) and that current fatality rate among SCT with IFI was 28% (9/32) although mortality in patients with IFI was 50%, higher than the non IFI cohort. Advances in clinical management, including anticipate diagnoses, a more appropriate use of antifungal drugs, and the presence of low numbers of really high-risk patients could be argued for explanation.

**0886 RANDOMIZED TRIAL OF PREVENTION OF CATHETER-RELATED BLOODSTREAM INFECTION IN PATIENTS WITH HEMATOLOGIC AND ONCOLOGIC DISEASE**

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**Backgrounds.** Data from the National Nosocomial Infection Surveillance system (United States) between January 1992 and February 1998 showed that Catheter-Related Bloodstream Infection (CRBI) is the third most frequent nosocomial infection and accounted for 14% of all nosocomial infections. CRBI may be caused by fibrin deposition associated with catheters. Interventions designed to decrease fibrin deposition have the potential to reduce CRBI. In a previous randomised study, we have shown that the use of continuous infusion of low-dose (100 IU/kg per day) unfractionated heparin (UFH) was a practical and economical approach to the prevention of CRBI in patients with hematologic-oncological disease. Aims. The purpose of this study was to evaluate the role of heparin-coated central venous catheter (CVC) in preventing CRBI in patients with hematopoietic-cell transplantation (HCT). Methods. This study was a randomised controlled trial in which patients were randomly assigned to receive either a heparin-coated CVC without a continuous infusion of low-dose UFH (heparin-coated group) or a non-coated CVC with a continuous infusion of low-dose UFH (control group). CRBI was defined

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according to the difference in time to positivity. Results. Between April 2005 and February 2006, one hundred and twenty patients were randomly assigned. Two patients were excluded after assignment. Ultimately, 118 patients were analysed. CRBl occurred in 5% (3 of 59 catheters) of those in the heparin-coated group (2.2 events per 1000 days) and in 8.5% (5 of 59 catheters) of those in the control group (2.9 events per 1000 days) (p=0.7). Two and three patients experienced severe bleeding in the heparin-coated and control groups, respectively (p=0.5). We did not observe heparin-induced thrombocytopenia. Conclusion. The use of heparin-coated catheter is a safe and effective approach to the prevention of CRBl in patients with hematologic and oncologic disease.

References


2. Abdelkefi A, Achour W, Ben Othman T, et al. Difference in time to positivity of the Fcγ receptor IIA, located mainly on neutrophils, strongly influence the binding capacity of the receptor, as the R variant binds to IgG2 poorly.


0887

POLYMORPHISMS OF MBL2, BUT NOT OF FCGRIIA AND MYELOPOEROSE PROMOTOR GENES, INFLUENCE THE RISK OF SEPSIS IN MULTIPLE MYELOMA PATIENTS DURING AUTOLOGOUS STEM CELL TRANSPLANTATION

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Background. A number of studies have indicated that polymorphisms of various immune defence genes interfere with the risk of severe infections in critically ill patients1-5 including studies in autologous6 and allogeneic6 stem cell transplantation. In MBL2 three well-known single nucleotide polymorphisms B (G54D), C (G57E), and D (R32C) (collectively termed O in contrast to the wild-type A) exist. Presence of O variants greatly reduces the efficiency functions of mannose-binding lectin - an important complement activating protein. The 131 H/R polymorphism of the Fcy receptor IIa, located mainly on neutrophils, strongly influence the binding capacity of the receptor, as the R variant binds to IgG2 poorly.6 The G'463A polymorphism of the myeloperoxidase promoter gene is very sparsely studied as a risk factor for infections, but carriers of the A variant possibly are at increased risk of infections during allogeneic stem cell transplantation.7 Aims. We aimed to examine the impact of the polymorphisms described above on the occurrence of severe infections related to ASCIT in patients with multiple myeloma. Methods. Patients were genotyped with PCR techniques using aliquots of peripheral stem cells from apheresis. Infectious complications were recorded retrospectively from clinical records and database extractions from the Department of Microbiology. This was done blinded to the genotypes. Results. One hundred and eleven consecutive patients were studied. During ASCIT 70 patients (63%) had fever above 38.5°C despite prophylactic antibiotics. Eleven (10%) patients had proven sepsis. MBL2 analyses: 4/71 patients with AA genotype had sepsis versus 7/40 with AO/OO genotype (p=0.09). Two lethal cases of sepsis were seen in the AO/OO patients, none in the AA patients. In multivariate analyses the risk of sepsis was significantly lower in AA patients: OR 0.15 (95% CI: 0.03-0.74), p=0.02. FCGRIIA and MPO promoter analyses: no association with fever or sepsis was found in patients with variant genotypes. Conclusion. Wild-type MBL2, associated with a high function of mannose-binding lectin, probably reduces the risk of sepsis in myeloma patients during ASCIT. Myeloma patients with variant MBL2 may be candidates for future MBL replacement trials. In this setting we cannot confirm the protective effect of wild-type FCGRIIA or 463MPO genotypes suggested earlier.8

References


0888

DEVELOPMENT OF A FAST BROADBAND SCREENING SYSTEM FOR BACTERIAL BLOOD STREAM INFECTIONS USING WHOLE BLOOD AND CULTURE FLASKS

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Backgrounds. Bacterial blood stream infections are a common side effect in immune suppressed patients like cancer patients. Normal microbiological routine diagnostics are time consuming and mainly based on classical cultivation. So far, molecular methods (e.g. PCR) were based on speciation of specific microorganisms like toxin-coding genes or species specific DNA fragments (e.g. 16S rDNA). We developed a novel extraction method where only bacterial DNA will be extracted and an amplification system that gives results within 2 hours. False positive results will be excluded and the infection causing organism will be identified at species level. Aims. Aim of this study was to detect the species of a bacterial blood stream as soon as possible after blood sampling. This may help to target an optimized antibiotic therapy and further medical treatments. False positive results had to be excluded. Methods. Blood of healthy donors and sterile culture flasks were spiked with various strains of bacteria known as causing clinical problems. The new extraction method only extracts the bacterial DNA. All human 'background' was digested. This increases the detection sensitivity and excludes false positive results attributed to cross reactions of bacterial universal primers with human mitochondrial DNA. By using PCR (Polymerase Chain Reaction) primers to amplify specific regions of the bacterial genome, PCR products vary in length specific to the amplified specific region. Each species gives a specific band pattern in the agarose gel after PCR-amplification. Results. Compared to commercial DNA-extraction kits, our extraction method shows a higher sensitivity and specificity when amplified directly out of whole blood. No false positive results could be detected, PCR gives results 2 hours after DNA extraction. Using realtime PCR, 10 bacterial cells per ml blood could be detected. Performing a PCR after cultivation in a culture flask, each positive flask gave a positive PCR result. Conclusion. Our results show that the species causing the bacterial blood stream can be identified directly out of whole blood or a culture flask without sequencing the PCR product or further selective cultivation steps. Within 3 hours after blood sampling respectively 2 hours after culturing in the flask, a species specific therapy can be performed. The risk of false positive results was eliminated.

0889

31P MRS IN VITRO ASSAY OF PHOSPHOLIPIDS FROM PLASMA, PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMC), AND BONE MARROW MONONUCLEAR CELLS (BMNC) OF PATIENTS WITH ACUTE LEUKEMIA (AL) AT THE TIME OF DIAGNOSIS

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Backgrounds. 31P NMR spectroscopy is convenient and precise tool for the phospholipid analysis of extracts from biological samples. Previous investigations in vitro were performed on tissue extracts of solid carcino-
mas: breast, esophagus, colon, as well as on plasma of patients with thyroid and kidney cancers, multiple myeloma, malignant lymphoma. *Aims.* The aim of this investigation was to examine: (a) whether 31P NMR spectra of phospholipid extracts from plasma, PBMC, and BMMC are suitable for the analysis of phospholipid metabolism of blast cells from patients with AL, (b) whether obtained spectra allow to differentiate lymphoblastic acute leukemia (ALL) from myeloblastic acute leukemia (AML). *Methods.* 31P MRS spectra were obtained from phospholipids extracts of plasma (21 healthy volunteers, 44 patients with AL), PBMC (11 healthy volunteers, 52 patients with AL), and BMMC (38 patients with AL). Cellular phospholipids were isolated from mononuclear cells (MC) by means of Ficol buffy coat centrifugation. Methanol-chloroform precipitation and extraction from 60k Ohm MC was performed according to the modified Folch's method. 31P MRS analyses were conducted on AMX 300 Bruker spectrometer 7.05 T. *Results.* 31P MRS spectrum of phospholipid extracts from normal human PBMC consisted of 6 peaks due to following phospholipids: phosphatidylcholine (PC), phosphatidylcholine plasmalogon (CPLAS), lyso phosphatidylcholine (LPC), sphingomyelin (SM), phosphatidylethanolamine (PE), and phosphatidylserine (PS). The peak due to LPC appeared only occasionally and not all spectra contained peak due to CL. However, the spectrum of phospholipid extracts from plasma consisted of 6 peaks due to phospholipids: PC, CPLAS, LPC, SM, PE, and PI. One peak due to MDPA was observed. Spectra obtained from phospholipid extracts from plasma and PBMC of patients with AL differed statistically from phospholipids of plasma and PBMC of healthy volunteers. Spectra obtained from phospholipid extracts of PBMC and BMMC patients with AL didn't differ. Spectra obtained from PBMC and BMMC of ALL patients differed significantly from AML patients. However, we did not observe any statistically significant difference within spectra from plasma (Table 1).

Table 1. Concentration of phospholipids.

<table>
<thead>
<tr>
<th></th>
<th>ALL (n=21)</th>
<th>AML (n=38)</th>
<th>ALL (n=52)</th>
<th>AML (n=44)</th>
<th>ALL (n=38)</th>
<th>AML (n=44)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC</td>
<td>2.01±1.58</td>
<td>1.62±1.06</td>
<td>0.32±0.18</td>
<td>0.40±0.18</td>
<td>0.24±0.16</td>
<td>0.42±0.26</td>
</tr>
<tr>
<td>CPLAS</td>
<td>0.05±0.08</td>
<td>0.06±0.06</td>
<td>0.02±0.03*</td>
<td>0.05±0.05*</td>
<td>0.01±0.02*</td>
<td>0.05±0.05*</td>
</tr>
<tr>
<td>LPC</td>
<td>0.08±0.11</td>
<td>0.07±0.09</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SM</td>
<td>0.50±0.43</td>
<td>0.42±0.32</td>
<td>0.04±0.05*</td>
<td>0.09±0.06*</td>
<td>0.02±0.04*</td>
<td>0.11±0.09*</td>
</tr>
<tr>
<td>PS</td>
<td>0.06±0.12</td>
<td>0.08±0.09</td>
<td>0.15±0.09*</td>
<td>0.28±0.17*</td>
<td>0.15±0.13*</td>
<td>0.30±0.19*</td>
</tr>
<tr>
<td>PL+PS</td>
<td>-</td>
<td>0.01±0.02*</td>
<td>0.04±0.04*</td>
<td>0.01±0.02*</td>
<td>0.04±0.04*</td>
<td></td>
</tr>
<tr>
<td>CL</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.03±0.00</td>
<td>0.01±0.03</td>
<td></td>
</tr>
</tbody>
</table>

Conclusion. Our data show that 31P MR spectra from PBMC and BMMC are identical, both in position of peaks and concentrations of phospholipids. It may indicate, that blast cells from PB demonstrate the same metabolism of phospholipids as blast cells from BM. Only in PBMC and BMMC of ALL patients differed significantly from AML patients. This may indicate, that blast cells from PB demonstrate the same metabolism of phospholipids as blast cells from BM. Only in PBMC and BMMC of ALL patients differed significantly from AML patients. How- ever, we did not observe any statistically significant difference within spectra from plasma (Table 1).

**0890**

**SAFETY OF A WEEKLY ADMINISTRATION OF 7.5 MG/KG OF LIPOSOMAL AMPHOTERICIN B FOR ANTIFUNGAL PROPHYLAXIS IN PATIENTS RECEIVING HIGH DOSE CORTICOSTEROIDS FOR ACUTE GVHD AFTER REDUCED INTENSITY CONDITIONING ALLOGENIC STEM CELL TRANSPLANTATION**

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RIC regimens are increasingly used for allo-SCT in elderly or patients not eligible for standard myeloablative allo-SCT. Such regimens have yielded promising results in terms of decreasing early transplant-related toxicities. However, acute GVHD remains a matter of concern in this setting. Of note, the use of high dose Cs for GVHD treatment increases the risk of severe fungal infections in this high risk population usually presenting several comorbidities. Therefore, prophylactic strategies aiming to reduce this risk are needed. We conducted a pilot single centre study in 15 adult patients receiving high dose Cs (2 mg/Kg/day) for acute GVHD therapy after RIC allo-SCT. Treatment consisted of a 2 hour weekly infusion of 7.5 mg/kg LAB with a maximum of 8 total doses. The primary endpoint was the incidence of serious adverse events occurring during the course of prophylaxis treatment. Of note, safety was monitored with particular attention to nephrotoxicity in this relatively elderly population receiving concomitant nephrotoxic drugs such as cyclosporin A. Median age of these 15 patients with various hematological and non-hematological malignancies was 54 years (range, 40-70). Patients received a median of 4 weekly doses of LAB (range, 1-8), with 8 patients (53%) receiving at least 4 consecutive weekly doses. In terms of toxicity, 6 patients (40%) didn’t experience any sign of toxicity. One patient experienced a violent chest pain with transient extra-systoles, during the first infusion of LAB, and did not receive any subsequent infusions. Other mild and transient infusion-related reactions (fever, flush, tachycardia, orthostatic hypotension, pruritus, bone pain, abdominal pain) were observed in 5 patients, usually at time of first LAB infusion. Despite concomitant administration of cyclosporin A in all 15 patients, only 4 patients (27%) had to interrupt the course of prophylactic LAB (respectively after 3 (n=2), 4 and 7 infusions) because of renal toxicity (increase of serum creatinine ≥1.5 times from baseline values). Although long term efficacy of such antifungal prophylactic strategy is yet to be established, the results of this feasibility study demonstrate that a weekly dose of 7.5 mg/Kg of LAB is relatively safe and well tolerated when given as prophylaxis in high-risk immuno-compromised patients receiving high dose Cs for GVHD after RIC allo-SCT, despite concomitant administration of multiple nephrotoxic drugs.

**0891**

**IMMUNITY AGAINST POLIO, DIPHTHERIA AND TETANUS AFTER CONVENTIONAL CHEMOTHERAPY TREATMENT FOR AML AND HIGH-GRADE LYMPHOMA**

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**Background.** In a recent paper, subprotective antibody levels against diphtheria and tetanus were found in a majority of pediatric patients after treatment for high-risk acute lymphatic leukemia (Ek L et al. 2005). Information is scarce regarding immune reconstitution in adult patients who have undergone chemotherapy treatment for hematological malignancies. *Aims.* The aim of this study was to investigate whether protective antibody levels against diphtheria, tetanus and polio are retained in adults after intense chemotherapy treatment of acute myelogenous leukemia (AML) and high-grade non-Hodgkin’s lymphoma (HGNHL). Patients and Methods. Thirty-two patients, 18 males and 14 females, median age 61 (19-79) years, all in CR1 for a duration of >6 months after conventional treatment for AML (n=16) or HGNHL (n=16), were included. Twenty-nine healthy sex-matched persons, median age 60 (24-69) years, were enrolled as a control group. Immunity against polio types 1, 2 and 3 were assessed utilizing a standard neutralization assay, whereas antibody levels against tetanus and diphtheria toxoids were determined using an ELISA and a neutralization test, respectively. The minimum protective thresholds, as defined for clinical samples by the microbiology laboratory, were used to categorize patients and controls as immune or susceptible to infection. Results. Subprotective antibody levels against at least one of three polio serotypes were found in 10 out of 32 patients (31%), to be compared with 2/25 (8%) in healthy controls (p<0.05, χ2 test). Twelve out of 32 (38%) patients versus 4/29 controls lacked immunity against diphtheria (p<0.05). With respect to immunity against tetanus, the difference between patients and controls (4/32 vs 1/29 pts) was not significant (p=0.36). Summary. We report a high prevalence of subprotective antibody levels against polio and diphtheria in AML and lymphoma patients who were in CR after conventional therapy not encompassing stem cell transplantation. Provided that these results can be confirmed in a larger study, assessment of immunity status and possibly vaccination, may be considered not only in patients with leukemia and lymphoma but also in other patient groups receiving intensive chemotherapy.
Background. Regular monitoring of the bacterial epidemiology at hemato logical units has been recommended in order to evaluate the effects of the prophylactic and empiric antibacterial strategies adopted. Aims. To observe the most frequent pathogens involved in infection complications and the emerging resistance to antibiotics. Methods. We analysed all the consecutive febrile/infectious episodes occurring to the 823 patients admitted to our Institution (248 Acute Leukemia, 233 Lymphoma, 195 Myeloma, 26 Myelodysplastic Syndrome, 28 Chronic Lymphocytic Leukemia, 10 Myeloproliferative Syndrome and 83 non neoplastic haematological patients) from June ’04 to September ’05. All the patients with expected neutropenia lasting for more than 7 days received prophylaxis with levofloxacin 500 mg/day. Results. Three hundred and forty-six patients developed fever/infection (44.2% of all admission) and in 183 episodes (51.6%) the Gram- infection was documented (bacteremia 46%, pneumonia 42%, urinary tract infections 7%, others 5%). One hundred and sixty-four patients were isolated in 157 microbiologically documented infections (37.6%) including 82 Gram- bacteria (50%), 66 Gram+ bacteria (40.2%), 14 fungi (8.5%) and 2 miscellaneous (1.5%). E.coli, Enterobacteriaceae other than E. coli, and Staphilococcus spp were the most common isolates, accounting respectively 25.2%, 9.8% and 10.4% of all isolates). Among Gram-, S. aureus, Coagulase-negative Staphilococci (CoNS) and Enterococci were the most frequent pathogens, accounting respectively 15.2%, 11% and 7.9% of all isolates. E. coli was statistically more frequent in patients affected by acute leukaemia (26/69, 38% vs 10/66, 15%, p<0.01), neutropenia <500/mm3 (31/81, 38% vs 7/56, 13%, p<0.01) and on prophylaxis with levofloxacin (26/66, 42% vs 8/43, 19% p<0.05) but not statistically different from the number of EPCs found in healthy volunteers (median 1,9 vs. 12,3 EPC-CFU/ml blood, p<0.001). We did not find any correlation between the number of EPC and the Birmingham Vasculitis Activity Score (BVAS), level of C-reactive protein, plasma creatinine or Activity Score. Patients with ANCA anti-PR3 antibodies had a trend for higher numbers of mature circulating endothe lial progenitor cells (EPC) compared to those with anti-MPO antibodies (median 0.18 vs 3.15 EPC-CFU/ml blood, p=0.08). The number of EPC in patients on long-term hemodialysis was also significantly lower in patients with AAV compared to healthy volunteers (median 0.5 vs 12.3 EPC-CFU/ml blood, p=0.001) and not statistically different from the number of EPCs found in patients with ACS. The details of interactions between endothelium, platelets, and leukocytes occurs in ACS, which involves the release of EMP and formation of EMP-monoocyte conjugates and PLC. These findings support prior studies suggesting that release of EMP and their binding to monocytes are key events in thrombogenesis. Our findings also support the concept that the formation of PLC regulates leukocyte activ ity and participates in linking thrombosis with inflammation.

Introduction. There are two essential types of endothelial cells circulating in blood. First type are mature endothelial cells (ECs), which numbers are increased in microcirculation disorders such as ANCA associated small vessels vasculitis (AAV). These cells are connected with vascular damage. The other type are circulating endothelial progenitor cells (EPCs), which originate from bone marrow and play a crucial role in vascular repair and cancer neoangiogenesis. ANA. As there are several works studying mature CEC in patients with AAV, we have explored the frequency of immature EPCs in these patients. Methods. Circulating EPC numbers were determined in 35 patients with AAV, including 16 patients with newly diagnosed active disease without prior immunosuppressive treatment, 15 patients with active disease already treated by immunosuppressive therapy (pulse i.v. cyclophosphamide and peroral corticosteroids) and 10 patients in remission of the disease. Six patients were investigated twice (at diagnosis and in remission). We have used three groups of controls: 15 patients with non-AAV renal damage (patients on long-term hemodialysis), 9 patients suffering from macrocirculation dis orders (ischemic disease of lower extremities) and 23 healthy volunteers. EPCs were enumerated by colony forming unit assay. 15-20ml of peripheral blood was centrifuged on Ficoll-Hypaque gradient (Pharma cia, Uppsala, Sweden) and mononuclear fraction was cultivated in the EndoCultTM medium (StemCell Technologies, Vancouver, Canada) according to manufacturer instructions. Clusters of at least 20 round cells surrounded by spindle-shaped cells were counted as endothelial precursor colonies. Results. The number of EPC was significantly lower in patients with AAV compared to healthy volunteers (median 0.5 vs 12.3 EPC-CFU/ml blood, p<0.001). We did not find any statistical difference in numbers of EPC among groups of AAV patients before and after beginning of treatment and a group of patients in remission on maintenance immunosuppression. We have also found no correlation between the number of EPC and the Birmingham Vasculitis Activity Score (BVAS), level of C-reactive protein, plasma creatinine or titre of ANCA. Patients with ANCA anti-PR3 antibodies had a trend toward lower numbers of EPC compared to those with anti-MPO antibodies (median 0.18 vs 3.15 EPC-CFU/ml blood, p=0.08). The number of EPC in patients on long-term hemodialysis was also significantly lower than in healthy volunteers (median 1.9 vs 12.3 EPC-CFU/ml blood, p=0.001) and not statistically different from the number of EPCs found in patients with AAV. Patients with macrovascular disorders had non-significantly lower numbers of EPC compared to healthy volunteers and significantly higher than patients with AAV (6.18 vs 0.5, p=0.035). CONCLUSION: Contrary to higher numbers of mature circulating endothelial cells, numbers of circulating endothelial precursors are significantly lowered in patients with ANCA positive vasculitis. This may reflect serious endothelial damage on one hand, coupled with diminished ability of endothelial healing. Immunosuppressive treatment, which is frequently also cytotoxic, may suppress not only the microvascular inflammation but also the endothelial healing process. Low numbers of EPCs in patients with terminal kidney disease may reflect the accelerated atherosclerosis found in uremia.
A RANDOMIZED COMPARATIVE STUDY OF RESPONSE TO EMPIRIC AMPHOTERICIN B DEOXYPHOSPHOCHOLATE ON DAY 4 OR DAY 8 OF FEBRILE NEUTROPENIA

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Background. Febrile neutropenic patients are at greater risk of getting bacterial and fungal infections. Initial therapy for these patients consists of broad spectrum antibiotics. Persistent fever without localisation in spite of more than 3 days of broad spectrum antibiotics including vancomycin qualifies for initiation of empiric antifungal therapy. However, the timing of initial empiric antifungal therapy can vary from 3 days to 8 days of non response to antibiotics. Aims. We choose to determine the response of empiric amphotericin B deoxycholate starting either on day 4 or day 8 in febrile neutropenic patients not responding to broad spectrum antibiotics and without localization of fever. The study also examined the side effects related to amphotericin B deoxycholate in this group of patients. Methods. Sixty five neutropenic patients with persistent fever despite 72 hours of antibacterial therapy were randomly assigned to receive amphotericin B either starting from day 4 (group A, n=27) or starting from day 6 (group B, n=29). Patients in both the groups were evaluated for efficacy and safety of study drug by the clinical criteria, frequent cultures, radiological procedures and laboratory parameters. A response was defined as satisfactory at the end of therapy if the patient was afebrile for 48 hours, had absolute neutrophil count (ANC) >0.5x10^9/L, and did not require study termination due to patient’s withdrawal from the study, drug toxicity, and persistent fever requiring changing of the therapy or death due to any case. Results. The median age of patients in group A and in group B was comparable (23 versus 25 years). There were 17 males in group A and 18 males in group B. The patient population consisting of acute myeloid leukemia, acute lymphoblastic leukemia, aplastic anemia, non Hodgkin lymphoma, Hodgkin disease, chronic myeloid leukemia and multiple myeloma was equally distributed in two groups. A satisfactory response occurred in 85.2% of patients in group A and 69.0% of patients in group B (p=0.090). Time taken for resolution fever was considerably less in group A as compared to group B (5.4±3.9 days versus 11.5±4.0 days, p=0.001) which was also reflected in total dose requirements between groups A & B respectively (592±288.4 mg versus 790.7±370.3 mg, p=0.028). The factors (age, sex, body mass index, baseline temperature, diagnosis, ANC) affecting the satisfactory response rate to amphotericin B were not statistically significant in two groups. Documented fungal infections were seen in 4 patients (14.6%) in group A as compared to 11 patients (37.9%) in group B (p=0.072). The adverse side effects of amphotericin B (nephrotoxicity, hypokalemia, hypomagnesemia) occurred at similar rates in the two groups. None of the risk factors studied (age, sex, body mass index, total dose of amphotericin B, baseline renal functions or exposure to various nephrotoxic antibacterial antibiotics) could be implicated in the causation of the reported adverse effect of amphotericin B. Conclusions. We conclude that initiating early empiric (day 4) amphotericin B deoxycholate in persistent febrile neutropenic patients leads to early response rate and decreased dose requirements of amphotericin B without increased risk of nephrotoxicity.

CAUSES OF INCIDENTAL NEUTROPENIA IN ADULTHOOD

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Backgrounds. The incidental discovery of neutropenia during routine blood counting represents a common problem for clinicians. However, there are no reported data of systematic evaluations of adults with incidental neutropenia. Aims. We aimed to identify the causes of incidental neutropenia in adults. Methods. Ninety-seven adults with incidental neutropenia were submitted to a clinical and laboratory approach, including complete and serial blood counts, direct and indirect antiglobulin test, bone marrow smear and biopsy, assessment of folate, vitamin B12 and iron status, serum liver enzymes, serum proteins, serological exams for hepatitis B and C virus, cytomegalovirus, mononucleosis, human immunodeficiency virus, antinuclear and anti-DNA antibodies and rheumatoid factor, dosage of free thyroid and thyrotropin, chest roentgenogram, and abdominal urography. The diagnosis of neutropenia due to exposure to chemical agents was defined in individuals with a occupational exposure to myelotoxic chemical agents and hypopcellularity of the granulocytic lineage in the bone marrow. The infectious, autoimmune, haematological and thyroid diseases, and nutritional deficiency-related neutropenia were defined when diagnoses of the diseases or condition were established by specific laboratory exams. The recovery of the neutrophil count with the resolution or control of the disease was also required for the identification of these categories of neutropenia. Ethnic neutropenia was defined in individuals of African ancestry, in whom neutropenia was also present in other relatives. Drug-related neutropenia was diagnosed in patients under treatment with drugs, in whom the neutrophil count reached the normal value when the treatment withdrawn. Cyclic neutropenia was used to refer to our paediatric patients, chronic neutropenic patients and the diagnosis of chronic idiopathic neutropenia of the adult (CINA) was established when none of the above mentioned causes was found. Results. CINA was identified in 34.0% of the individuals, neutropenia due to exposure to chemical agents in 14.5%, infectious diseases in 9.3%, autoimmune diseases in 9.3%, haematological diseases in 9.3%, thyroid disorders in 8.2%, ethnic neutropenia in 7.2%, drug-related neutropenia in 2.1%, cyclic neutropenia in 2.1%, and iron deficiency in 2.1%. Recovery or improvement of the neutrophil count was seen upon treatment or recuperation from infectious, autoimmune, haematological and thyroid diseases, and iron supplementation. Conclusions. We conclude that the evaluation of individuals with incidental neutropenia using

THE NEURONOPATHIC SPECTRUM OF GAUCHER DISEASE: A SINGLE-CENTRE CLINICAL EXPERIENCE WITH 15 PAEDIATRIC PATIENTS

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Backgrounds. Gaucher disease patients are deficient in activity of the lysosomal enzyme glucocerebrosidase. As a consequence, accumulation of glucocerebroside occurs in macrophages in tissues throughout the body. An immunologic disease manifestations develops in part as a consequence of secondary damage. In neuronopathic Gaucher disease, the central nervous system is also affected. Aims. We studied the clinical characteristics observed in a cohort of paediatric chronic neuronopathic Gaucher patients under our care in order to delineate the spectrum of systemic and neurological manifestations. The data were compared with clinical data collected from another cohort of 20 paediatric patients with non-neuronopathic (type 1) Gaucher disease. Methods. All 15 neuronopathic patients were thoroughly assessed at an initial evaluation (detailed history, physical examination, assessment of developmental status, and measurement of haemoglobin, platelet count and biochemical disease markers). Spleen and liver volumes were assessed (sonography) and chest X-ray, plain X-ray of the pelvis and DEXA scanning of the distal ulna were performed. Patients underwent comprehensive neurological examination, including evaluation of strabismus, eye movements and brain auditory evoked potentials (BAEP). Results. Compared to our non-neuronopathic patients, chronic neuronopathic patients had significantly earlier diagnosis, significantly greater anaemia, and higher serum chitotriosidase activity, as well as ACE and lysozyme levels. Most of our patients with severe neurological involvement had pronounced splanchnomegalgy in conjunction with bone and lung manifestations. A markedly higher degree of radiological evidence of pulmonary interstitial involvement and higher frequency of skeletal complaints were apparent. A considerable variety in types and combinations of neurological symptoms was observed. Prominent neurological abnormalities with early development of a saccade initiation failure was ubiquitous in our series and a combination of strabism and saccade initiation failure was common. BAEP abnormalities were also common. A large percentage of our patients had severe neurological disease with hypereflexia and ataxia and multifocal and/or myoclonic epileptic manifestations. Strabism as the first clinical neurological manifestation were detected in approximately half of our patients. Summary/Conclusions. The majority of our neuronopathic Gaucher patients first had systemic manifestations of the disease and after disease development neurological abnormalities as the first neurological symptom. Therefore, we recommend that objective eye movement assessment is carried out in all children diagnosed with Gaucher disease. Presentation and progress of neuronopathic disease was remarkably variable and, in fact, each patient is unique throughout the rigid historical categorization. Neuronopathic Gaucher disease variants is still used by many, we are of the opinion that it fails to express the variability. Systemic disease presentations in chronic neuronopathic Gaucher represented a more severe, early progressive condition than in type 1 Gaucher.
a structured approach may possibly identify the identification of clinically
silent diseases, and provide the opportunity for early treatment, avoid-
ing complications of the diseases and consequences of neutropenia.

0898
LONG TERM FOLLOW-UP OF TYPE 1 GAUCHER DISEASE PATIENTS. - A RETROSPECTIVE ANALYSIS
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Santa Maria Hospital, Lisbon, Portugal

Background. Gaucher disease (GD) is a rare familiar disorder. It is
caused by mutations in the glucocerebrosidase gene that causes a defi-
ciency in β-glucocerebrosidase activity. In result, cleavage of glucose
from ceramide is impaired and glucose-ceramide accumulates within
cells. Three clinical phenotypes are recognized. Type 1 is the non-neu-
rologic variant commonly seen in Ashkenazi Jews, but also found in
other ethnic populations. Type 2, the infantile neuropathic form, and
type 3, the juvenile onset variety, cause neurologic deterioration and are
much rarer. Aims. Considering our hospital is a reference centre for GD
in Portugal, our aim is to revise our Type 1 GD Patients data and report
on diagnosis, treatment and long term follow-up results in order to
enhance the understanding of this rare disease and to assess patients and
their response to therapy over time, with the ultimate goal of improv-
ing the clinical outcomes through the definition of specific strategies.
Methods. The total GD Type 1 patients (14) diagnosed at our outpatient
department was enrolled to this retrospective analysis. All but 2 patients
have therefore been on ERT for at least 1 year and the longest follows-
up is 9 years. Amongst our group,12 patients were allocated to ERT
(Enzyme Replacement Therapy) and the remaining 2 to SRT (Substrate
Reduction Therapy); according to the approved indication. ERT (IV infu-
sion treatment) initial loading dose was 60 U/Kg every two weeks except
for one patient whose disease severity leaded to a 120 U/kg dose to
start with. SRT (oral treatment) dose was 100mg TID. Results. The exam-
ined criteria for all patients included treatment related improvement
(symptoms, physical examination, haematological parameters and bone
disease) and Serious Adverse Events (SAEs) incidence. We found only
one patient to be positive to Imiglucerase antibodies although there was
no related clinical impact. This analysis indicated that ERT was well tol-
erated, we had no SAEs reported concerning both symptoms and labo-
atory parameters and the main issue turned out to be treatment adher-
ence specifically related to infusions. Due to SAEs, SRT had to be dis-
continued to one of the treated patients and later on it was decided to
initiate ERT. Conclusions. In respect to ERT, this study confirms the lit-
erature data concerning clinical improvement and good tolerance. At
this time point, we have no sufficient data to draw conclusions on SRT
and the patient on treatment is apparently doing well. In conclusion, the
methodology adopted at our centre for this patient population has to be
considered as appropriate; furthermore, ERT impact on disease para-
eters also enables us to assess compliance; considering patients’ health
status interest, the level of drug investment and the future consequences
of disease progression, we are currently analyzing patient profiles and
motivations in order to identify positive strategies to raise treatment
(intusion) adherence.

0899
THE SAFETY AND EFFICACY OF CONTINUOUS DYNAMIC DOSE ANTICOAGULATION DURING CHEMOTHERAPY-INDUCED THROMBOCYTOPENIA
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Background. The optimal management of patients with haematologi-
cal malignancies who require therapeutic anticoagulation for throm-
boembolic disease or prosthetic cardiac valves while receiving myelo-
suppressive chemotherapy has not been established. In particular, the
role of anticoagulation during chemotherapy-induced thrombocytopenia,
with its attendant increased risk of bleeding, has not been prospectively
assessed. Aims. To assess the safety and efficacy of a dynamic dosing
strategy for continuous anticoagulation during chemotherapy-induced
thrombocytopenia. Methods. Patients were treated between January 2000
and January 2006 at The Alfred Hospital, Melbourne; prospective assess-
ment occurred for patients treated between January 2005 and January 2006.
All were receiving myeloablative chemotherapy for a haematologi-
cal malignancy, and required anticoagulation for radiographically proven
venous thrombosis or prosthetic cardiac valves. Patients were anticoagu-
lated with subcutaneous enoxaparin as follows: 1 mg/kg body weight
twice daily (Full dose) while the platelet count was ≥20x10^9/L and 0.5 mg/kg
once daily (Reduced dose) while the platelet count was <20x10^9/L. Enoxaparin was withheld if the platelet count could not be
supported above 20x10^9/L; if there was bleeding; and for 12 hours prior
to and following procedures. Platelet were transfused to maintain a count ≥20x10^9/L. Results. Ten patients were enrolled prospectively, and 45
assessed retrospectively. In 54 patients, the indication for anticoagulation
was venous thromboembolic disease; the other had a prosthetic aortic
valve. Three underwent allogeneic stem cell transplantation, and seven
autologous transplantation; the remainder received other myeloablative
multi-agent chemotherapy regimens. Detailed information regarding the
delivery of anticoagulation was available for patients enrolled prospec-
tively. The median number of days of thrombocytopenia <150x10^9/L was
19 (range 9-40). Enoxaparin was delivered at full dose on 31% of throm-
boocytopenic days, at reduced dose on 63% of days, and withheld on 6%
of days. Of the days where enoxaparin was withheld, 45% were the
result of procedures; 20% bleeding; and 35% other reasons, including
major bleeding rate occurred in 5.4% of all patients. In the prospective group, three patients experienced episodes of minor bleeding. No major bleeding was observed in this group.
In the retrospective group, two patients developed major gastrointestinal
bleeding requiring endoscopic intervention and transfusion while receiv-
ing reduced dose anticoagulation with platelet counts of 20/50x10^9/L. One
developed bleeding requiring surgical intervention at a wound site when
the platelet count was 19x10^9/L, and enoxaparin had been withheld. Data
regarding minor bleeding episodes were not available for patients in the
retrospective group. No thromboembolic complications were identified in
either group. Conclusions. The strategy employed here was not associated
with excess bleeding. It was effective in preventing recurrence and exten-
sion of thromboembolic disease in patients with a range of haematologi-
cal malignancies. We conclude that dynamic dose anticoagulation may be
delivered safely and effectively during chemotherapy-induced thrombo-
cytopenia.

0900
SURVIVAL IN MULTIPLE MYELOMA PATIENTS IS NOT AFFECTED BY DEVELOPMENT OR RECURRENCE OF THROMBOEMBOLISM
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Backgrounds. Patients with cancer who developed venous thromboem-
bolism have a poor prognosis. Aims. We have analyzed the effect on
survival of treatment-associated VTE and their recurrence in a homoge-
eous population of myeloma patients enrolled on our Total Therapy 2
protocol. Methods. 668 newly diagnosed myeloma patients were enrolled in a study, which included induction phase with VAD, DCEP,
CAD, and DCEP followed by tandem high dose chemotherapy and
peripheral blood stem cells (PBSCT) transplants. Patients were randomly
assigned upfront to receive Thalidomide or not. Both arms received
identical chemotherapy. Patients were followed and when clinically
indicated underwent radiological studies to confirm a suspected VTE.

Thrombosis II
Concurrent aspirin or other anticoagulants were not given. Pts who were receiving prophylaxis with low-dose warfarin were evaluable. Warfarin for the prophylaxis of VTE in MM pts treated with T containing regimen. Different approaches for T associated VTE prophylaxis have been such as dexamethasone (D), anthracyclin (A) or platinum compounds. VTE is a common and often problematic event remains unknown, the optimal method for prophylaxis is also different in those who resume thalidomide. Conclusion: Our experience shows that a thromboembolic event during treatment for myeloma patients does not affect survival.

**0901 LOW-DOSE WARFARIN DECREASES THE INCIDENCE OF TALIDOMIDE ASSOCIATED VENOUS THROMBOEMBOLISM IN PATIENTS WITH MULTIPLE MYELOMA**

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Introduction. T and its analog lenalidomide have established their role as novel antineoplastic agents. VTE is a common and often problematic side effect of these agents. The incidence of VTE increases substantially (up to 26%) when T is combined with other therapeutic agents such as dexamethasone (D), anthracyclin (A) or platinum compounds. Different approaches for T associated VTE prophylaxis have been explored without any consensus to date. Since the underlying pathologic event remains unknown, the optimal method for prophylaxis is also undefined. We prospectively investigated the use of low-dose warfarin for the prophylaxis of VTE in MM pts treated with T containing regimen. Methods. All pts treated with T containing regimens at our institute receiving prophylaxis with low-dose warfarin were evaluable. Warfarin was given at doses 1 or 2 mg for actual body weight of < 70kg or > 70 kg respectively, which is continued for the duration of the T treatment. Concurrent aspirin or other anticoagulants were not given. Pts who were fully anticoagulated for a prior VTE or other VTE risk factors are excluded from this analysis. Results. Eighty-two consecutive MM pts, median age 60 years (range 43-82) who received low-dose warfarin for T containing regimen are reported here. T was given in combination with either dexamethasone, VAD regimen, Bortezomib (B) or bortezomib/doxil. Of these 38 are male and 44 female. Median dose of T was 200 mg per day (range 50-800). Duration of therapy with T varied with the regimen used with a maximum duration of 6 months. Of these pts, 54 (68%) received T with D containing regimen, 56 (68%) with A and 31 (38%) with B containing regimen. Some pts received multiple T containing therapies during their clinical course. Four (4.8%) out of 82 pts were noted to have a VTE. Conclusion. Although we continued to see VTE with T, our experience suggests that low-dose warfarin can effectively decrease the overall incidence of VTE in pts treated with T containing regimens. Interestingly, none of the pts who received T with doxil experienced any episode of VTE. This finding warrant further investigation of low-dose warfarin as VTE prophylaxis for T based therapies.
incidence of clinically overt CVC-related thrombotic complications in patients with hematological malignancy is not negligible. The thrombosis of the lumen of the catheter is the frequent complication of central vein cannulation. However their necessity of catheter removal is negligible. Although symptomatic disease was not developed in our cases of subclinical thrombosis, doppler-ultrasound screening may be useful to identify the patients with subclinical thrombosis that require antithrombotic treatment.

0904
DOPPLER ULTRASOUND ASSESSMENT OF SUBCLAVIAN VENOUS BLOOD-FLOW AFTER THE IMPLANTATION OF A CENTRAL VEIN CATHERETER IN CHILDREN WITH MALIGNANCY
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1Połtawian Medical University, SZCZECIN, Poland; 2Medical University, SZCZECIN, Poland

A central vein cannulation is a routine procedure in the management of children with cancer. This long lasting, permanent venous access, resulting in a significant improvement of the quality of life of these patients is usually achieved by the implantation of a tunneled central venous catheter (CVC) into one of the subclavian veins. The undisputable benefit of this procedure is limited by its side effects, necessitating a long follow up of larger groups of patients. The clinical relevance of these changes remains unknown and requires a long follow-up of larger groups of patients.

0905
TIME COURSE OF INFLAMMATION AND PROTHROMBOTIC PARAMETERS IN ACUTE CORONARY SYNDROME
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1Hospital U. Rio Hortega, Valladolid, Spain; 1Icior, Valladolid, Spain

Background. The aim of this study is to assess the dynamics and magnitude of the thrombosis plasma markers in peripheral blood, and the relation to the degree of the inflammatory response across the spectrum of the acute coronary syndrome. Methods. Fifty patients with acute coronary syndrome; 10 with ST elevation myocardial infarction (STEMI), 10 patients with non-ST elevation myocardial infarction (NSTEMI), 10 patients without stable angina (UA), 10 patients with stable angina (SA) and 10 comparable healthy controls were enrolled. The values of von-Willebrand factor antigen (vWF), thrombin-antithrombin complex (TAT) and prothrombin fragment 1+2 (F1+2) were assessed in peripheral blood using an enzyme linked immunoassay method. The samples were collected on admission, daily during the first week, and days 14, 21 and 30 after the coronary event.

The inflammatory response was determined by a C reactive protein for every patient at the same times. Kruskal-Wallis test and Spearman’s Rho test were used to establish relationship between these markers. Results. There were an increased prothrombotic response associated to myocardial damage, the values of vWF, TAT and F1+2 in patients with acute coronary syndrome were increased since the first day from the onset of symptoms, peaking on 4th or 5th day, and verifying in the table until 1 month. There were significantly higher levels in patients with myocardial infarction related to the other diagnoses (<0.01), specially at admission and at peaking plasma levels of these parameters (table 1). There were positive correlations between vWF & CPR (r = 0.67), TAT & CPR (r = 0.59) and F1+2 & CPR (r = 0.78), Statistical significance was determined by using the Wilcoxon test. Conclusions. The study demonstrates a marked prothrombotic response across the spectrum of acute coronary syndrome with correlation to the myocardial damage. The inflammatory response is closely associated with the prothrombotic response.

0906
ELECTIVE SURGERY IN ANTAGOCOAGULATED ATRIAL FIBRILLATION PATIENTS WITHOUT BRIDGING THERAPY WITH LMWH
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Hospital de Laredo, LAREDO (CANTABRIA), Spain

Backgrounds. When oral anticoagulant therapy are discontinued for surgery, low-molecular- weight heparin (LMWH) is often used as bridging therapy. However, this practice has never been evaluated in randomized clinical trials. In our Hospital, the bridging therapy with LMWH is only used in prosthetic valves or embolism high risk atrial fibrillation patients. Aims. To assess the efficacy and safety of discontinuing anticoagulation in embolism low risk atrial fibrillation patients without bridging therapy. Methods. Retrospective observational study, from January 2000 to March 2005. Oral anticoagulant therapy was stopped 4 days before the elective surgery in atrial fibrillation patients without previous cardioembolism episode. If INR was >1.5 on the day of surgery, parenteral anticoagulation was considered. When it was possible, oral anticoagulant was restarted the evening of surgery. Venous thrombosis prophylaxis was performed, when necessary, with dalteparin (5000 IU daily) starting 12 hours before surgery and until the INR was >1.9. Medical records (including emergency attendances) were reviewed to check arterial embolism or bleeding episodes until 90 days after surgery. Results. 150 surgeries were eligible from 130 patients (75 men) with a median age of 76 years-old (44-91) and at least one of the following embolism risk factors: mitral valve disease, age >75, hypertension, diabetes mellitus or cardiac insufficiency. The majority procedures were orthopaedic surgeries (95%), followed by orthopaedic, digestive system, urologic, gynaecological and otolaryngology surgeries (31, 24, 20, 9 and 1 respectively). 74% of the procedures were ambulatory major surgery, 25% major and 3% minor surgery. No surgery was postponed. In 35% of cases venous thrombosis prophylaxis was made. One patient (0.5%; 95% CI, 0 to 1 1) had a transient ischemic attack 21 days after surgery and 2 patients (1.1%; 95% CI, 0.4 to 1.8) had an episode of bleeding (one mild metrorrhagia after gynaecological surgery and one acute cerebral hemorrhage 2 months after surgery). Conclusions. The arterial embolism ratio in our study is similar to the reported in anticoagulated patients when LMWH is used as bridging therapy. Oral anticoagulation treatment in atrial fibrillation patients without previous cardioembolism episode could be discontinued for surgical procedures without using LMWH bridging therapy. This strategy simplifies the perioperative management of anticoagulation in these patients, reduces the cost and avoids LMWH adverse effects without increasing the embolic risk.

Table 1. Median values at admission (a) and maximum level (b).

<table>
<thead>
<tr>
<th></th>
<th>ITCa µg/L</th>
<th>TATb</th>
<th>F1+2a nmol/L</th>
<th>F1+2b</th>
<th>VWFa %</th>
<th>VWFb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.2</td>
<td>3</td>
<td>0.48</td>
<td>0.5</td>
<td>101.5</td>
<td>106.5</td>
</tr>
<tr>
<td>UA</td>
<td>2.9</td>
<td>3</td>
<td>0.59</td>
<td>0.64</td>
<td>111.5</td>
<td>114</td>
</tr>
<tr>
<td>STEMI</td>
<td>2.5</td>
<td>15.4</td>
<td>0.75</td>
<td>1.12</td>
<td>102.3</td>
<td>138</td>
</tr>
<tr>
<td>NSTEMI</td>
<td>7.6</td>
<td>14.1</td>
<td>1.03</td>
<td>1.66</td>
<td>140</td>
<td>153.5</td>
</tr>
</tbody>
</table>

NOTE: all P values were <0.05.
VALIDATION OF THE COMPUTERIZED DECISION SUPPORT SOFTWARE TAOCHECK TO MONITOR ORAL ANTICOAGULANT THERAPY

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Backgrounds. Many clinical trials have demonstrated the utility of computer-based dosage programs to monitor oral anticoagulant therapy (OAT) in outpatients. However some of them are not already validated. Aims. We carried out a prospective, randomized trial to validate the effectiveness of the computer application Taochek (Roche Diagnostics). Methods. From March to June of 2004, 118 outpatients on OAT in maintenance phase (more than 3 months under OAT) were randomized to two groups: 56 patients into the experimental group (Taochek-aided dosing) and 62 into the control group (experienced physician dosing). There were not differences between two groups regarding age, sex, and diagnosis to AOT. Patients did not know the allocation. Dosing recommendations made by Taochek could be overridden by a physician. The comparison between both groups was performed by individual proportion of time spent within the therapeutic target INR range and the INR range ±0,3 by the mid-interval step method. Results. There were no differences regarding the time in INR or INR±0,3 range between groups: The patients of both groups spent the 50% of time into the INR target range (p=0.64); Taochek group spent the 81.6% of time of into the INR±0,3 target range vs 94.4% of control group (p=0.599). The number of appointments varied in both groups, the number of appointments was 30 days in the Taochek group was higher than in the control group (median 1.4 vs 0.9; p<0.001). From a total of 129 determinations in the Taochek group, 6 times (5%) the dose was not proposed by the software or was overridden by the physician and in 5 time (4%) the dose was not proposed the first day schedule dose was modified. Conclusions. Our study demonstrated that OAT can be provided at least as well by computerized decision support software Taochek as by experienced physician. However the Taocheck-aided dosing group required more number of appointments per patient than the control group. Taocheck software is useful for the AOT control and offers an effective help to inexperienced clinical staff.

D-DIMER SAMPLES

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D-dimers are used to assist diagnosis of venous thrombo-embolism (VTE), but are often inappropriately requested and interpreted. We retrospectively reviewed 4606 serial d-dimer (DD) requests. We documented indication for request, repeat requests and compared results at different levels of raised DD. Our current DD test-kit is auto dimer (Trinclus). The frequency of raised DD ranged from 20% (460) to 3% (98) and 6% (288) at 5 days apart. In 2 patients, the test was requested (and performed) on 3 samples on the same day. In only one instance did a repeat produce a different result, in a borderline positive case which became negative. These samples represent a very small percentage of the total workload, but were clearly not indicated. As is well-known, DDs rise during pregnancy and pregnant women often suffer dyspnoea, leg swelling or musculo-skeletal chest pain. Of 71 pregnant or postnatal women with symptoms and raised DD in all groups, only 2 had a confirmed VTE. In summary, DD is over-requested in our institution; grossly elevated DDs are used to contrast hypothesis. There were no statistically significant correlations between antiplatelet drugs resistance and neither the plasma concentration of HCY nor the C807T GPla polymorphism. 1. Platelet aggregation studies reveal resistance to aspirin and clopidogrel therapy in relatively few patients after myocardial infarction. 2. Platelet C807T glycoprotein Ia (GPla) polymorphism is common among referred individuals. Apart from platelet glycoprotein IV (GPIV), GPla is known as the main platelet subendothelial collagen receptor. The aim of the study was to find a relation between asp. and clopidogrel resistance and GPla polymorphism. Material and methods. The study group consisted of 50 patients aged 40-76 (median 57.2): 35 men (70%) and 15 women (30%). 32 of them had positive family history of coronary disease. All patients had a diminished intraplatelet concentration of malonylaldehyde (MDA) due to ASA ingestion. Resistance to antiplatelet drugs has been determined by the following criteria: the intensity of platelet aggregation induced by ADP >60%, by collagen >70%, and by arachidonic acid (AA) >20%. Platelet C807T glycoprotein Ia polymorphism has been detected by allelespecific PCR/RFLP method (Santoso S. et al.). Homocysteine (HCY) concentration in the plasma has been evaluated by HPLC.

Table 1.

<table>
<thead>
<tr>
<th>The frequency of aspirin and clopidogrel resistance (platelet aggregation)</th>
<th>Hyperhomocysteinemia</th>
<th>Polymorphism C807T GPla</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADP</td>
<td>Collagen</td>
<td>Arachidonic acid</td>
</tr>
<tr>
<td>3.5µM</td>
<td>5µM</td>
<td>2µg/mL</td>
</tr>
<tr>
<td>Moderate</td>
<td>Medium</td>
<td>Heterozygote</td>
</tr>
<tr>
<td>(platelet aggregation)</td>
<td>16-30µM</td>
<td>30-100µM</td>
</tr>
<tr>
<td>5(10%)</td>
<td>9(18%)</td>
<td>3(6%)</td>
</tr>
</tbody>
</table>

There were no statistically significant correlations between antiplatelet drugs resistance and neither the plasma concentration of HCY nor the C807T GPla polymorphism. Conclusions. 1. Platelet aggregation studies reveal resistance to aspirin and clopidogrel therapy in relatively few patients after myocardial infarction. 2. Platelet C807T glycoprotein Ia (GPla) polymorphism and moderate hyperhomocysteinemia are very common in survivors of myocardial infarction. 3. There is no interrelationship between resistance to aspirin and clopidogrel therapy and platelet C807T glycoprotein Ia polymorphism or homocysteine plasma concentration.
NUMBER AND MIGRATORY ACTIVITY OF CIRCULATING ENDOTHELIAL PROGENITOR CELLS IN PATIENTS WITH VENOUS THROMBOEMBOLISM

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Backgrounds. Endothelial progenitor cells (EPC) derived from bone marrow are believed to support the integrity of the vascular endothelium. The number and function of EPC correlate inversely with cardiovascular risk factors, but the prognostic value associated with circulating endothelial progenitor cells has not been defined. Aims. We hypothesized that altered EPC biology may contribute to the pathophysiology of venous thromboembolism (VTE). Methods. EPCs were determined in patients with VTE (n=52) and in a normal control group (n=47) by fluorescence-activated-cell sorting (FACS) analysis. Cells that were positive by flow cytometry for CD34/KDR/AC133 within the lymphocyte population were characterized as EPCs. Results. Patients with VTE showed markedly decreased numbers of EPC (45.7%) and colony forming units (74.8%) when compared with the controls (p<0.001). These findings were corroborated by 29.8% decrease in EPC migratory function in response to vascular endothelial growth factor (VEGF) (p=0.039) and 47.6% decrease in EPC incorporation into human umbilical vein endothelial cells (HUVEC). In addition, framingham’s risk factor score of both HIV-infected patients (r=0.472, p=0.01) and normal group (r=0.376, p=0.012) significantly correlated with the numbers of EPC. Indeed, the number of circulating EPC was significantly lower in patients with VTE than in normal group under the same burden of risk factors (p<0.001). Conclusions. EPC biology, which is critical for neovascularization and the maintenance of vascular function, is altered in patients with VTE. Our data strongly suggest that dysfunction of circulating EPC has a role in the progression of cardiovascular complications in these patients.

SIGNIFICANCE OF PLASMA THROMBIN-ACTIVATABLE FIBRINOLYSIS INHIBITOR LEVELS IN PATIENTS WITH VENOUS THROMBOEMBOLISM

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Backgrounds. Recently, a new potent inhibitor of fibrinolysis, the thrombin-activatable fibrinolysis inhibitor (TAFI), has been isolated from human plasma. The possibility that TAFI also participates in the mechanism of hypofibrinolysis has not been appraised in patients with venous thromboembolism (VTE). Aims. In the present study, we investigated the plasma levels of TAFI and its relation to urinary albumin excretion in patients with VTE with normo- and microalbuminuria. Methods. Forty-seven patients with VTE (29 with normoalbuminuria, 18 with microalbuminuria) and 45 age-matched normal subjects were enrolled in this study. Results. The plasma level of thrombin-antithrombin complex was significantly increased (25.2±2.4 vs. 7±1.3 nmol/liter; p<0.05), whereas the D-dimer/thrombin-antithrombin complex ratio was significantly decreased (14.6±1.2 vs. 25.4±2.7; p<0.05), showing the occurrence of hypercoagulability and hypofibrinolysis in patients with VTE. The plasma level of TAFI in VTE was significantly elevated, compared with normal subjects (156.1±10.3 vs. 97.4±4.5%; p<0.05). The plasma level of TAFI in patients with microalbuminuria was significantly higher than the level in those with normalalbuminuria (185.2±23.4 vs. 179.9±11.9%; p<0.05) or normal subjects (188.6±23.4 vs. 98.7±4.3%; p<0.01). Univariate analysis showed that the plasma TAFI levels are significantly and proportionally correlated with urinary albumin excretion (r=0.47, p<0.05), and with plasma soluble thrombomodulin level, a marker of endothelial cell damage, in all patients with VTE (r=0.47, p<0.05). Conclusions. These data suggest that increased plasma level of TAFI may be involved in the mechanism of vascular endothelial damage in venous thromboembolism.

SOLUBLE P-SELECTIN LEVELS IN DIABETES MELLITUS PATIENTS WITH CORONARY ARTERY DISEASE

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Backgrounds. Type 2 (non-insulin-dependent) diabetes is associated with a marked increase in the risk of coronary heart disease. Platelets play a significant role in coronary artery disease. Soluble P-selectin is an index of platelets activation. Aim of the Work: is to assess the soluble P-selectin levels in Coronary artery disease. Aims: to evaluate the levels of P-selectin in coronary artery disease order to determine its clinical significance. Methods. Soluble P-selectin levels were measured by ELISA in the peripheral blood of 55 diabetic patients with coronary artery disease (21 acute myocardial infarction (AMI), 20 with unstable angina (UA), 14 with stable angina (SU)), 20 patients with diabetes mellitus without coronary artery disease (DM without), and 10 healthy controls. Results. Soluble P-selectin level was significantly higher in patients with AMI (M±SD: 239.3±13.0 ng/mL), than those with UA (141.5±15.2 ng/mL), SU (92.1±7.67 ng/mL), DM without (89.6±7.1 ng/mL), and healthy control (69.1±4.5 ng/mL) (p<0.001). In patients with US, sP-selectin was found to be significantly elevated as compared to the SU, DM without and control group. sP-selectin was not significantly different in DM without as compared to controls. The sP-selectin levels was correlated to the duration of diabetes mellitus (R=0.35, p=0.03). Moreover, sP-selectin level was significantly higher in AMI patients with recurrent anginal attack as compared to that in those with single attack (p<0.041). Multivariate analysis revealed that sP-selectin levels at presentation had high adverse influence on coronary artery insult compared to LDL cholesterol level, degree of hypertension. Conclusion. Measurement of soluble p ’selectin level may be helpful marker of impending coronary artery insult in diabetic patients.
Background-Aims. Platelet plays a crucial role in the pathogenesis of arterial occlusive disorders and platelet-dependent thromboembolism is considered an underlying mechanism in the pathogenesis of Coronary Artery Disease (CAD). The glycoprotein (GP) Ia/Ila, also known as integrin α2β1, is an important mediator of the adhesion of platelets to fibroblast collagen. Several case-control studies have investigated the importance of the α2 gene (GPia) C807T polymorphism, a genetic marker of integrin α2β1 surface levels, as a risk factor for CAD, but the research findings were controversial. CAD continues to be the major cause of morbidity and mortality in the Western world. Hence, we carried out a meta-analysis in order to determine the importance of this polymorphism as a risk factor for CAD. Methods. Nineteen studies with data on the contribution of GPia C807T polymorphism to coronary risk were identified through a comprehensive MEDLINE search up until October 2005. The random effects meta-models were performed with data on studies that specifically examined cases with CAD including Myocardial Infarction (MI) and patients with only MI (including those with Acute Coronary Syndrome (ACS)). Results. The C versus the T allele contrast in the CAD group yielded an OR of 0.998 (95% CI: 0.997-1.004). The combined estimate was also insignificant when we performed the analysis in studies involving cases either with MI or with an ACS (OR: 1.013; 95% CI:0.942-1.089). Similarly, comparing the C with the T homozygotes in the CAD group, we derived a non significant OR (OR, 1.054; 95% CI:0.898-1.236) and all other comparisons (CC genotype versus the others or TT genotype versus the rest) did not suggest any gene-disease association. There was no between studies heterogeneity and publication bias might have not influenced the magnitude of the effect. The results remained unaffected when we fitted meta-regression models including variables such as age, risk level, gender, geographical origin, and smoking habits. Conclusions. We failed to show that the C807T polymorphism of the α2 gene could influence susceptibility to CAD either as an independent factor or in combination with any conventional risk factor.

Background-Aims. Platelets are crucial in primary haemostasis and their adhesion to damaged vessel wall is mainly mediated by the collagen receptor glycoprotein (GP) Ia/Ila, known as integrin α2β1. Besides limiting blood loss at sites of tissue trauma, platelet thrombi are also responsible for the obstruction of diseased vessels, resulting in ischemia and infarction of vital organs. Although the C807T single nucleotide polymorphism of integrin α2 gene (GPia) correlates with increased platelet surface levels of the integrin α2β1, the results concerning the association of this genetic variant with ischemic stroke have been controversial. In order to clarify this association, we performed a meta-analysis of published data regarding this issue. Methods. Seven studies providing data on the contribution of GPia C807T polymorphism to the development of ischemic stroke were found through PubMed search. For the analysis of data, we used random effects models and meta-regression. Results. The pooled frequency of the T allele was 36.33% in cases and 37.01% in controls, with the T versus the C allele contrast gave an OR of 1.11 with a 95% CI 0.827-1.499. Furthermore, comparing the T homozygotes with the C homozygotes, we derived a non-significant OR (OR, 1.36; 95% CI: 0.637-2.887). Similarly, the two other contrasts (CC genotype versus the others or TT genotype versus the rest) provided absolutely no evidence of any gene-disease association. There was significant between study heterogeneity (p<0.05). In one of the contrasts, the difference in males’ percent between cases and controls was significant in the meta-regression suggesting an improper matching with regard to sex. Conclusions. This meta-analysis failed to show any significant influence of the 807T allele on the risk of stroke neither in the group of patients as a whole nor in any relevant subgroup. However, due to the significant diversity between a small number of studies in the present meta-analysis, the interpretation of the summary effect has to be done with caution.
to girls. A strong relationship between b-TG, atheromatous index, PAs and triglycerides was also found in children with positive family history of CAD. Conclusion: Platelets activation takes place during childhood in children with family history of CAD. Those findings suggest that the inflammatory process of atherosclerosis begins early in childhood in individuals whose parents have developed prematurely coronary artery disease.

**0918**

**SERUM LEVELS OF SOLUBLE E-SELECTIN IN VENOUS THROMBOEMBOLISM (VTE)**

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Background. The inflammatory response of the vein endothelium seems to have relevance in the acute phase of the venous thromboembolism (VTE) and involves a strong expression of some cell adhesion molecules (CAMs). The serum levels of the soluble form of the E-selectin (sE-selectin) are related with the activation of endothelial cells but the evolution of their values in a later (chronic) phase of the VTE is not known. We aim to identify any association between the sE-selectin and the VTE six months after the acute phase. Population and Methods. We measured the serum sE-selectin concentrations from 394 subjects. 197 control subjects were patients objectively diagnosed of venous thromboembolism (VTE) six months after the acute phase and 197 controls of similar gender and age [61.5(12.9) years, 49.0% males] by an enzyme-linked immunosorbent assay (ELISA) method. The non-Gaussian distribution of the sE-selectin values requires the use of non-parametric statistical tests. Results. In the overall series, the level of the sE-selectin directly correlates with the waist/hip ratio (r = 0.21, p < 0.0001) being higher among the males [66.0(54.5) vs. 55.5(58.1) ng/mL, p < 0.001] although showing a weak inverse correlation with the age (r = -0.135, p < 0.01). The sE-selectin was independent of the body mass index (NS p). A trend to lower sE-selectin values appears among the patients [56.8(42.6) ng/mL] versus the controls [65.0(47.6) ng/mL] (p = 0.055, Mann Whitney test). However the extreme values did not show association with the VTE [90th percentile (124.5 ng/mL); OR = 1.06, NS p and 10th percentile (33.1 ng/mL); OR = 1.17, NS p]. The soluble E-selectin was also similar in recurrent versus the controls [66.0(54.5) vs. 55.5(38.1) ng/mL, p = 0.05]. Ninety-nine (94.3%) compliant patients. Conclusions. In patients with VTE, higher homocysteine levels are not associated with higher levels of thrombin generation markers and homocysteine reduction by B-vitamin supplementation had no effect on these markers.

**0920**

**IMPACT OF A DIAGNOSTIC PROTOCOL FOR DEEP VEIN THROMBOSIS ON REQUESTS FOR D-DIMER ASSAYS**

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Backgrounds. Sensitive D-dimer assays have been shown to be useful for decreasing the need for formal radiological imaging in the diagnosis of Deep Vein Thrombosis (DVT) in selected patients who have low clinical pretest probability (PTP) scores. Aims. Unfortunately in our institution the PTP score was not always assessed and the D-dimer test was therefore being used to rule out DVT even in those patients who would have had a high PTP. This is inappropriate and has resulted in missed diagnoses. Methods. In Jan ‘05 we introduced a diagnostic protocol in A+E, which relied on a Wells’ PTP Score (Figure 1). Patients with low PTP (0 or 1) and D-dimer value < 250 ng/mL did not require radiological imaging and were discharged. The haematology laboratory was empowered to reject samples for D-dimer testing if the request form did not contain a Wells’ PTP. In contrast, patients with a high PTP (2 or 3) proceeded to radiological imaging without a D-dimer test. An audit of compliance with the new pathway was assessed, and an action plan formulated to promote awareness and compliance. Results. The results were compared one year later: the number of D-dimer requests has decreased by 50% with the new protocol. There are now no apparent missed DVTs (as indicated by patients who returned to the A+E department and later were confirmed to have DVTs in the year prior to introduction of the protocol). Summary: D-dimer assays must be used in conjunction with a clinical pre-test probability score; a low PTP and a negative D-dimer can reliably exclude DVT. Prior to the introduction of this protocol we received 350 D-dimer requests per month. The introduction of a Wells’ PTP and selective use of D-dimer testing resulted in cost savings, BMS time efficiency and significant relief in manpower pressures in providing the 4-hour D-dimer service.

References

1. BCSH. BJHaem, 124,15-25
**0921**

**RELATIONSHIP BETWEEN HOMOCYSTEINE LEVELS AND MTHFR GENOTYPE, AND THEIR EFFECT ON DEEP VENOUS THROMBOSIS**

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**Backgrounds.** High serum total homocysteine concentrations (tHcy) are suggested to be a risk factor for arterial and deep venous thrombosis. 5,10-methylenetetrahydrofolate reductase (MTHFR) is a crucial enzyme in homocysteine metabolism. The mutation MTHFR C677T renders the enzyme thermolabile and leads to elevated tHcy levels. Aim: In this study the relationship between tHcy levels and MTHFR C677T polymorphism in patients with first episode of deep venous thrombosis (DVT), as well as the assessment of hyperhomocysteinemia as risk factor for DVT were investigated.

**Methods.** 46 (20 men, 26 women) healthy individuals (group A) aged 21.52±14.11 years and 74 (37 men, 37 women) patients with first episode DVT (group B) aged 45.99±14.98 years were enrolled. Total homocysteine levels were determined with ACS: 180 SE Automated Chemiluminescence Systems (Bayer). MTHFR genotypes were analyzed using PCR amplification and digestion with restriction endonuclease Hinf I. The data were expressed as the mean ± SD and analyzed with Student test, Univariate Analysis of Variance, Tukey test, Logistic Regression Analysis. Values of p<0.05 were considered to indicate statistical significance.

**Results.** Hyperhomocysteinemia was set at 90th percentile of tHcy levels of group A. Results. Statistically significant difference in tHcy levels between groups A and B was observed (A vs B, 12.44±4.48 vs 14.54±5.37 µmol/l, p=0.029). The frequency of alleles was 0.598/0.669 for C allele and 0.402/0.311 for T allele in groups A and B, respectively. Among the subjects with T/T genotype, higher tHcy concentrations were detected in group B than group A (T/T, A vs B, 13.07±4.67 vs 22.47±7.22, p=0.012). No important difference was found in the tHcy levels between the two groups with respect to C/C (p=0.141) and C/T (p=0.392) genotype (A vs B, C/C 11.28±4.17 vs 13.07±3.96, C/T: 13.14±4.55 vs 14.60±5.16). There was no effect of MTHFR C677T mutant genotype on tHcy levels in group A. Total Hcy concentrations in patients (group B) with T/T genotype are statistically higher when compared to C/C (C/C vs T/T, 13.07±5.96 vs 22.47±7.22, p<0.001) and to C/T (C/T vs T/T, 14.60±5.16 vs 22.47±7.22, p=0.001) genotypes. Hyperhomocysteinemia (tHcy > 19.30 µmol/l) was observed at 7.9% (4/46) of group A and 21.6% (16/74) of group B. Logistic regression analysis indicated that only hyperhomocysteinemia is an independent risk factor for DVT (Odds Ratio=3.95, CI 95%: 1.1-14.4, p=0.037), while the genotype (p=0.268) and the interaction between genotype and hyperhomocysteinemia (p=0.568) are not risk factors. Conclusions. Our results indicated that patients with T/T genotype have higher tHcy levels when compared to healthy individuals as well as to patients with C/C and C/T genotypes. Hyperhomocysteinemia is an independent risk factor for deep venous thrombosis. The T/T genotype and the combination of hyperhomocysteinemia and T/T genotype are not related to deep venous thrombosis.

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**0922**

**ADAMTS-13 GENE MUTATION IN A PATIENT WITH THROMBOTIC THROMBOCYTOPENIC PURPURA (TTP)**

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**Background.** von Willebrand factor (vWF) cleaving protease (ADAMTS-13) is important for maintaining the normal size distribution of vWF multimers. A severe deficiency of ADAMTS-13 activity (i.e. <5% of that of normal plasma), caused by either mutation of the ADAMTS-13 gene or by inhibitory auto-antibodies to ADAMTS-13, is associated to TTP. Aim and Methods. We describe the case of a 19-year-long chronic relapsing TTP. A 45-year-old woman was first diagnosed with TTP, during pregnancy, in 1987 when she was 27 years. Subsequently, she relapsed three times in 1995, once in 1996, and once in 2000. On each episode she was treated with plasma exchange and steroid therapy, obtaining the complete remission. Results. In 2001, the ADAMTS-13 testing became available in our laboratory and we prospectively followed the patient for ADAMTS-13 activity and inhibitors. In addition, since 2005, we also tested the plasma levels of anti-ADAMTS-13 antibody. In 2004 she relapsed after starting interferon for HCV-related chronic hepatitis. Plasma exchange and steroid therapy were resumed, without achieving a durable remission, as she relapsed after one month. She was then given chemotherapy (i.e. fludarabine, cyclofosfamide) and rituximab, without significant response. From January 2005 on, she is receiving periodic plasma infusion on the basis of platelet count and is continuing on this regimen so far. The measurement of ADAMTS-13 activity from 2001 showed a severe deficiency (<5%) of this protease during clinical remissions and upon relapses. No significant plasma inhibitory activity was found by mixing studies. The retrospective quantification of auto-antibodies by ELISA revealed no significant levels of anti-ADAMTS-13 antibodies. The patient was then identified as a possible carrier of a true constitutive ADAMTS-13 deficiency. The DNA analysis of this patient detected homozygosity for the 5428 C>T in exon 25 of the ADAMTS-13 gene, which predicts the R1123C exchange in the TSP1-8 domain. The inherited nature of severe ADAMTS-13 deficiency was established by family analysis. Conclusions. This mutation has been previously linked to Upshaw-Schulman syndrome, which characterized by neonatal onset, response to fresh plasma infusion, and frequent relapses. Differently, in our patient the onset of clinically overt disease manifested in the adult age during pregnancy, thus supporting the hypothesis that additional precipitating factors may determine the phenotypic manifestation of this mutation.

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**0923**

**IS THE VARIABLE CLINICAL PRESENTATION IN HEREDITARY TTP THE RESULT OF DIFFERENCES IN RESIDUAL ADAMTS13 ACTIVITY?**

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**Backgrounds.** Hereditary TTP (Upshaw Schulman syndrome, USS) is due to homozygous or compound heterozygous ADAMTS13 gene mutations. Analysis of patient histories revealed a striking age-dependency of the first TTP attack. While half of the patients develop clinical signs of acute TTP immediately after birth or in early childhood (early onset), the other half remains asymptomatic into early adulthood and suffers from a first acute TTP episode at the age of 20-40 years (late onset) and this clinical pattern is often very similar in affected siblings. So far, no correlation between the clinical phenotype, i.e. disease severity, organ tropism and the underlying genotype is discernable. In analogy to the situation in hemophiliacs (distinction between <1% and 1-5% determination of residual ADAMTS13 activity could help to elucidate this variable clinical presentation). As vWF levels increase with age a critical threshold might eventually be exceeded so that these patients’ residual ADAMTS13 activity is no longer sufficient to prevent TTP bouts. Methods. Index patients from 28 families with USS were included into this study. Only plasma samples withdrawn at least 4 weeks...
after the last plasma infusion were analyzed. ADAMTS13 activity was determined by a new fluorescence resonance energy transfer assay using a synthetic, truncated 73-amino-acid VWF peptide as a substrate (FRET-VWF73 assay; Kokame et al. Br J Haematol. 2005;129:95-100), which was modified in order to reliably distinguish between 0% and 1% of ADAMTS13 activity, which had not been possible with the older assays. Results. Half of the U53 patients (14/28) had an ADAMTS13 activity <1%, but FRET-VWF73; 11 patients displayed a residual activity between 1-5% and in three instances ADAMTS13 activities lay between 5-8%. Attempts to link patients histories with their ADAMTS13 activities failed, as patients from both clinical groups (early vs. late onset) had ADAMTS13 activity values <1% or in the range of 1-5%. Conclusions. Differences in residual ADAMTS13 activity are apparently not accountable for the documented age-related presentation in USS. It is thus likely, that other disease-modifying genetic (i.e. blood group, VWF levels) or environmental factors affect the phenotype.

**0924 INCIDENCE AND LABORATORY FEATURES OF THROMBOCYTOPENIA IN 43 PATIENTS WITH VON WILLEBRAND DISEASE TYPE 2B: CORRELATION WITH MOLECULAR DEFECTS AND ACQUIRED MODIFICATIONS OF VWF**

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Backgrounds. Von Willebrand type 2B (VWD) is an inherited bleeding disorder caused by abnormal von Willebrand factor (VWF) that displays increased affinity to the platelet glycoprotein 1b-α (GpIbα). VWD 2B is due to a group of mutations clustered within VWF A1 domain and is characterized by binding of its high molecular weight multimers (HMW) to platelets often resulting in moderate-mild thrombocytopenia. Even though there are many case reports on thrombocytopenia associated with VWD 2B, retrospective and prospective studies in a large cohort of patients are not available. Aims and design of the study. To determine incidence and laboratory features of thrombocytopenia in VWD 2B, we have prospectively observed our cohort of 43 patients (18 families) previously characterized by VWF mutations. Methods. Data of platelet count with mean platelet volume (MPV) and morphologic evaluation of the blood smear to search for giant platelets or aggregates were associated with the history of physiologic or pathologic stress conditions such as pregnancy, infections, surgery or use of DDAVP. All patients were characterized by ristocetin induced platelet agglutination (RIPA) in the Platelet Rich Plasma (PRF), ristocetin cofactor activity (VWF:RCO) with VWF antigen (VWF:Ag), multimeric structure of VWF. Mutations within VWF A1 domain were searched for and confirmed by sequencing exon 28.

### Table 1.

<table>
<thead>
<tr>
<th>Mutation</th>
<th>RIPA VWF:Ag Basal Post MPV gp/aggreg. Plt.morphology</th>
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<tbody>
<tr>
<td>R1306W(15)</td>
<td>0.85</td>
</tr>
<tr>
<td>R1308C(5)</td>
<td>0.72</td>
</tr>
<tr>
<td>R1308L(5)</td>
<td>0.50</td>
</tr>
<tr>
<td>I1309V(6)</td>
<td>0.40</td>
</tr>
<tr>
<td>I1318H(3)</td>
<td>0.50</td>
</tr>
<tr>
<td>I1337L(4)</td>
<td>0.50</td>
</tr>
<tr>
<td>I1341Q(4)</td>
<td>0.67</td>
</tr>
<tr>
<td>R1341W(1)</td>
<td>0.70</td>
</tr>
</tbody>
</table>

Results. Among 43 VWD cases, a platelet count <140,000 was found at baseline in only 11 (26%), but was observed after stress conditions in 34 cases (79%); no reduced platelet counts was found in 9 patients (21%) from two different families (R1308L, R1341Q). An increased MPV was found in 35 cases but giant platelet and aggregates in only 6 cases. All the phenotypic features were correlated to VWF mutations. Conclusions. Based on these results, thrombocytopenia can be associated in most VWD 2B patients, especially when high levels of mutant VWF are triggered by physiologic and pathologic stress conditions. However, not all VWD 2B show thrombocytopenia and a relatively high degree of heterogeneity of this phenomenon occurs within patients characterized by the same molecular defects.

**0925 DIFFERENT ACTIVATION STATUS IN PERIPHERAL VERSUS SPLENIC T LYMPHOCYTES IN IMMUNE THROMBOCYTOPENIC PURPURA PATIENTS**


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Backgrounds. Adult idiopathic thrombocytopenic purpura (ITP) is an autoimmune disorder characterized by enhanced splenic destruction of the platelets, through autoantibodies binding to membrane glycoproteins. B lymphocytes secreting antplatelet antibodies are considered as the major mechanism in ITP. Nevertheless, cellular immunity could have a role in ITP pathophysiology. Recently, autoreactive CD4+ T cells directed against membrane platelet proteins have been identified and another group pinpointed the implication of CD8+ cytotoxic T lymphocytes for a direct cell-mediated lysis of autologous platelets in active ITP. Aim: We explored activation status of both CD8+ and CD4+ T-lymphocyte subsets in chronic ITP patients in peripheral blood and spleen when splenectomy was indicated. We surmised that different T-lymphocyte activation status could reflect different pathogenic mechanisms involved in platelet destruction. Methods. Fifty four patients with chronic ITP were enrolled prospectively in the present study and compared to 46 normal healthy volunteers. Among ITP group 15 patients had a splenectomy. Phenotypical analysis was done on ITP splenic T-lymphocytes and compared to T-lymphocytes obtained from post traumatic splenectomy. Flow cytometry was used to evaluate T-lymphocyte HLA-DR membrane expression. We used Wilcoxon-Mann-Whitney test to compare continuous variables, as they did not present normal distributions. We performed a Bonferroni adjustment to prevent the raise type I errors due to multiple testing between groups. Results. All 46 patients fulfilled ASH criteria for chronic ITP. Their ages ranged from 16 to 79 years with a median age of 49 years. Sixteen patients were male and 38 female. Median platelet count was 425,000/mm3 (1000-156,000/mm3). The percentage of CD8+DR+ peripheral T-lymphocytes was significantly higher in ITP patients (10.79% vs 7.20% p=0.004), with predominance for activated CD4+ subsets (6.12 vs 2.71 p=0.0001) compared to activated CD8+ (7.6 vs 5.36 p=0.0045). This activation was correlated with platelet counts for both subsets (p<0.05) (Figure 1).

Nevertheless, this activation status was not correlated to treatment efficacy nor prognosis. Study of splenic T-cell subset activation reveals different results. Indeed, only CD8+CD8+ splenic lymphocytes were found activated in ITP spleen, compared to controls (14.5% vs 4.96-36.65) vs 7.12% vs 5.95-13.27) p=0.000. Conclusion: From the present study we can conclude that there is a correlation between the severity of ITP and the increased percentage of activated T lymphocytes. Nevertheless, there is no correlation between this activation and prognosis. Interestingly, refractory ITP are characterized by an increase of splenic CD8+ T lymphocytes that could be involved in platelet destruction. This observation may corroborate the possible implication of different pathogenic pathways involved in ITP.
0926
PREVALENCE AND RELEVANCE OF HEPARIN-INDUCED ANTIBODIES IN LMWH-TREATED PREGNANT WOMEN
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Backgrounds. Heparin-induced antibodies (HI-Ab) and heparin-induced thrombocytopenia (HIT) have been demonstrated less frequent due to LMWH than UFH, however, this has been questioned in non-surgical patients more recently. So far no HIT cases in LMWH-treated pregnant women have been reported, whereas thrombocytopenia of other aetiology may develop during pregnancy. Aims. The purpose of this study was to investigate whether women treated with LMWH during pregnancy present with or develop HI-Ab and whether HI-Ab are of clinical relevance.

Methods. 111 women with a history of thrombosis (n=71), risk factors for thrombosis (n=40) and/or recurrent fetal loss (n=19) completed 121 pregnancies and were treated with LMWH or danaparoid (nadroparin n=101, dalteparin n=9, enoxaparin n=9, certoparin n=6, danaparoid n=5) during pregnancy and for 4 to 6 weeks after delivery. Inherited thrombophilia was diagnosed in 68/111 (61.3%) patients, 40 of them (59%) had experienced thrombosis. LMWH was initiated between week one and 30 of pregnancy depending on the defect and thrombosis risk. Development of HI-Ab was investigated by heparin-platelet-factor-4-ELISA (Asserachrom HPIA, Roche, Germany) in 4-8 week intervals. Positive ELISA results were additionally tested by the heparin-induced platelet-activation-assay (HIPPA). Platelet count was monitored once a week during the first 6 weeks of LMWH treatment and thereafter every 4-8 weeks. All measurements of HI-Ab were performed after termination of heparin treatment unless platelet count dropped for more than 50% or thrombocytopenia (< 150 G/l) developed. Results. HI-Ab were detected in 6/121 (5.0%) pregnancies by ELISA, none was positive in the HIPPA. Four of the six patients had a history of previous UFH exposure. Four patients had low (OD <1.0) and two intermediate HI-Ab-titers (OD 1.0-2.0; Cut-off OD 0.43-0.67 depending on the batch). In two patients with low titres (OD 0.63 and 0.56) HI-Ab were already present before LMWH treatment and normalised in one during LMWH administration. Interestingly, two patients with repeated pregnancies revealed HI-Ab during the first but not the second pregnancy. None of the six patients with HI-Ab developed thrombocytopenia or a platelet drop >50%. However, in 8/115 (7.0%) pregnancies without HI-Ab mild thrombocytopenia (range 105-147 G/l) became apparent mostly in the third trimester. Local allergic reactions occurred in 7.2% of patients and required change of anticoagulation.

Conclusions. Heparin-induced antibodies in pregnancies treated with LMWH are detectable with low frequency and usually low titres. Antibodies might in part be due to previous UFH exposure. None of the HI-Ab-positive patients developed thrombocytopenia, hence, the risk of LMWH-induced thrombocytopenia in pregnant women appears to be very low. Most thrombocytopenias are pregnancy-associated and of mild type, however might be difficult to distinguish between heparin and other factors as cause.

0927
INCREASED PLATELET-MONOCYTE AND PLATELET-NEUTROPHIL COMPLEX FORMATION IN PRIMARY RAYNAUD PHENOMENON AND IN RAYNAUD PHENOMENON SECONDARY TO SYSTEMIC SCLEROSIS
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Backgrounds. Although it was suggested that platelet activation in systemic sclerosis (SSc) was secondary to microvascular damage, there is also data that it is the primary event. As there is both platelet and leucocyte activation in both primary raynaud phenomenon (RP) and RP secondary to SSc, it is possible that increased platelet-leucocyte interaction contributes to coagulation system alterations in RP patients. It was stated that platelet-leucocyte interaction was an important factor in the pathogenesis of vascular ischemic syndromes. In addition, active platelets secrete microparticles (PMP) with procoagulant activity. This is the first study to evaluate platelet-monocyte complexes (PMC), platelet-neutrophil complexes (PNC), and PMP in RP. Aims. We evaluated platelet activation markers and PMP, PNC in patients with primary RP and in RP secondary to SSc. Methods. We utilized whole blood flow cytometry to quantify the expression of CD62P, PMP, and the percentages of PMC and PNC in primary RP patients and in SSc patients with secondary RP. Results. We included 16 consecutive SSc patients with sec-

ondary RP (15F, 1M, mean age: 44.3), 12 primary RP patients (10F, 2M, mean age: 38.6), and 18 healthy subjects (16F, 2M, mean age: 39.2) as our control group. The mean duration of symptoms in primary RP patients was 7.2 years, and it was 8.4 years in patients with secondary RP. CD62P expression in SSc patients with secondary RP was significantly higher than in primary RP patients and in controls (p values, respectively, 0.017 and 0.001). PMC and PNC in the other SSc groups were significantly higher in both primary and secondary RP groups than in controls (all p values <0.001). Although PMP level in primary RP group was higher than in controls, this difference was not significant (p=0.1). Table 1. PMC level in SSc patients with pulmonary artery hypertension (PAH) was significantly higher when compared to those without PAH (4±0.5 vs. 3.4±0.6, p=0.049). The other parameters evaluated in SSc patients did not significantly differ between groups with or without digital ulcers or loss, those with or without interstitial lung disease, aspirin-users and nonusers (p>0.05). In addition, PMP level had a correlation with pulmonary artery pressure (r=0.59, p=0.017). There was a trend towards higher PMP levels in the anti-centromere-positive group (4.1±0.4 vs. 3.5±0.8, p=0.1). In primary RP patients, PMC level had positive correlations with PNC (r=0.68, p=0.015) and CD62P (r=0.61, p=0.035). In SSc patients with secondary RP, PMC level had a positive correlation with PNC (r=0.68, p=0.001); CD62P level had a negative correlation with PMP (r=-0.5, p=0.046). In 4 patients administered iloprost, the mean CD62P level decreased significantly (16±7.4 vs. 10.4±3.9, p=0.03); PMC (62±20 vs. 50±10) and PNC levels (34±11 vs. 27.5±6.5) regressed nonsignificantly (p values >0.05). Conclusion. Our results suggest that platelet-leucocyte complex formation is increased in RP. In addition, it provides evidence that there is ongoing platelet activation and platelet-leucocyte interaction which might be due to anti-platelet therapy. It is important to consider as it might have potential therapeutic implications with respect to the use of antiplatelet drugs in these patients.

Table 1. CD62P,PMC,PNC,PMP in RP patients.

<table>
<thead>
<tr>
<th></th>
<th>Primary RP</th>
<th>RP secondary to SSc</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD62P (%)</td>
<td>6.7±3.8</td>
<td>17.7±13.9</td>
<td>6.1±8.6</td>
</tr>
<tr>
<td>PMP (%)</td>
<td>67±20.1</td>
<td>64.1±27.3</td>
<td>22.4±9.1</td>
</tr>
<tr>
<td>PNC (%)</td>
<td>37.2±22.8</td>
<td>33.3±16.6</td>
<td>9.2±4.1 PMP (%)</td>
</tr>
<tr>
<td>4.2±1.4</td>
<td>3.7±0.7</td>
<td>3.5±0.5</td>
<td></td>
</tr>
</tbody>
</table>

CD62P: SSc is different from in primary RP and controls (p values 0.017 and 0.004); PMC: primary and SSc are different from controls (p values <0.001); PNC: primary RP and SSc are different from controls (p values 0.001 and <0.001).

0928
LOW RATE OF LONG LASTING REMISSEMS AFTER SUCCESSFUL TREATMENT OF IMMUNE THROMBOCYTOPENIA WITH RITUXIMAB
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Backgrounds. Immune thrombocytopenic purpura is characterised by peripheral platelet destruction due to autoantibodies derived against surface glycoproteins. Management of patients with autoimmune thrombocytopenias is difficult, relapses are common. Recent studies have shown that the anti-CD20 antibody rituximab is effective in the treatment of relapsed and refractory patients. Aims. The aim of this study was to evaluate rituximab therapy in ITP patients in our institution. Methods. We report the results of a retrospective analysis of rituximab treatment in 14 patients with immune thrombocytopenic purpura. All patients had received 1-7 lines of previous therapies, 4 had undergone previous splenectomy. Rituximab was administered at the standard dose of 375 mg/m² once per week with a median of 4 infusions (range 1-4). Results. The overall response rate was 64%, 7 of 14 patients (50%) achieved a complete remission (platelet levels >100x10⁹/L). 2 of 14 patients (14%) showed a partial remission (platelets >50x10⁹/L). 5 patients did not respond to the therapy. The median time to response after the start of the rituximab treatment was 4 weeks (range 1-4). Three patients (21%) had a long lasting remission up to 156 weeks. Responding patients remained in remission for a median period of 8 weeks (range 10 days - 36 months). All of the 4 splenectomized patients had a complete remission after rituximab therapy, with 2 long lasting remissions for 26 and 156 weeks. Summary/Conclusions. Our observations show that rituximab treatment represents a well tolerated and effective therapy for patients with autoimmune thrombocytopenias even in previously refrac-

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IPT is an autoimmune disease characterized by increased anti-platelet autoantibodies, which bind to circulating platelets resulting in their destruction by the reticuloendothelial system. Despite its clinical importance, the diagnosis of IPT is one of exclusion, thus, inevitably associated with potential difficulties. CD40 is a cell surface receptor that belongs to the tumor necrosis factor receptor (TNF-R) family, and that was first identified and functionally characterized on B lymphocytes. CD40-ligand (CD40L) on CD4+ T lymphocytes, respectively, were measured using the technique of flow cytometry. An antigen-specific assay for platelet-associated antibody CD154 on CD4+ T lymphocytes and for CD40 on CD19+ B lymphocytes, respectively, were measured using the technique of flow cytometry. The surface expression of plt receptors was determined by flow cytometry using the following monoclonal antibodies: anti-CD41 (Gp IIb/IIIa) FITC and PE, anti-CD62p (p-selectin) PE, anti-CD11b FITC, anti-CD145S (Immunotech, Marseille, France) and PAC-1 (activated Gp IIb/IIIa) FITC (BD Biosciences, San Jose, CA, USA). The samples were analyzed on a Coulter® EPICS XL-MCLTM. All parameters were collected in list mode and the mean fluorescence units (MFI) of total plt population. CD62p and leukocytes positive for plt were expressed in percentage (%). Descriptive statistics and correlation test were also studied. Results. Blood samples from 3 females and 22 males with a median age of 40 years (range: 25-61) were studied. See descriptive statistics in table below. We found a significant correlation coefficient (r=0.837) between P-selectin expression and PAC-1 binding. We didn’t find a correlation between age and activation parameters. Conclusions. 1) Our data indicate that ADP induced plt activation varies considerably from one individual to one, as observed by other groups. 2) In healthy adults we demonstrate that the expression of P-selectin (α granules release) is strongly correlated to the binding of PAC-1 (conformational change) according to the results of other series.

| Table 1 |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| PAC-1 ADP (%)   | 0.4             | 0.3-0.7         | 0.1             | 0.1             | 25              | 9.7-51.9        | 11.4            |
| CD62p (MFI)     | 11.2            | 4.4-18.0        | 3.2             | 11.2            | 8.5             | 3.5-38.6        | 7.6             |
| CD62p ADP (%)   | 26              | 11.3-57.4       | 11.2            | 11.2            | 17.3            | 8.5-58.5        | 14.5            |
| CD62p ADP (%)   | 14              | 1.7-44.1        | 10              | 1.7             | 8.7             | 0.8-44.5        | 10.1            |

**0931 EFFICACY AND SAFETY OF IVIG3I GRIFOLS (HUMAN INTRAVENOUS IMMUNOGLOBULIN) IN PATIENTS DIAGNOSED WITH IMMUNE THROMBOCYTOPENIC PURPURA**

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Intravenous immunoglobulin (IVIG) therapy is an useful treatment in patients with chronic idiopathic/immune thrombocytopenic purpura (ITP) in whom the platelet count has to be rapidly increased to prevent bleeding or prior to surgery. IVIG3I Grifols is a highly purified, unmodified human IgG product whose manufacturing process follows the same basic principles of HebroY.31 (another IVIG manufactured by Grifols in clinical use since 1992). The main differences between both processes are how purification steps are sequentially arranged, and the introduction of two specific steps to inactivate/remove any potential contaminating pathogen (solvent-detergent treatment and sequential nanofiltration through 35 and 20 nm pore size filters), as additional viral elimination steps to pasteurization, already present in HebroY.32 Essentially, IVIG3I Grifols is prepared from fraction II-III of Cohn’s fractionation and the purification of IgG is performed by means of sequential polyethylene glycol precipitations. Further reduction down for all remaining potential impurities is achieved through ion exchange chromatography with DEAE resins. Finally, it is formulated with sorbitol (5%) as stabiliser. An open, prospective, multicentre study was planned to investigate the efficacy and safety of IVIG3I Grifols in 20 adult patients with chronic ITP (at least 6 months after diagnosis). It was designed in accordance with the European Union guidelines from the EMEA for such trials. A total of 19 adult patients with chronic ITP in acute phase (platelet counts below 20×10^11/L) were treated with the study drug. Patients received a total dose of 0.4 g/kg body weight for 5 consecutive days. Primary efficacy endpoint was the proportion of patients who reach a platelet count equal or > 50×10^11/L. The time taken for the platelet count to reach the target level since first dose of IVIG3I Grifols and duration of response
were also determined within 1 month after first infusion. Regression of haemorrhages was documented during the first 14 days of follow-up. Safety parameters including adverse events (AEs), laboratory determinations and vital signs were regularly monitored. The follow-up of patients ended 3 months after first dose of IGVISI Grifols to determine any change in viral markers for HIV, HCV, HBV and HAV. An interim analysis from available results of 8 treated patients presented a total of 12 AEs potentially related to the study drug (8 mild and 4 moderate). Headache and fever (4 cases each), changes in blood pressure (3 cases) and decrease in heart rate (1 case) were AEs potentially related to study drug. The results show that IGVISI Grifols is effective, well tolerated and safe in the treatment of adult patients with chronic ITP.

**0932**

**RITUXIMAB IN REFRACTORY IDIOPATHIC THROMBOCYTOPENIC PURPURA**


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**Background.** Rituximab, a chimeric anti-CD20 monoclonal antibody effective in B-cell depletion, may be useful in autoimmune disorders by interfering with the production of auto-antibodies. **Aims.** To investigate the effect of Rituximab in patients with refractory ITP. **Patients and methods.** Eleven adult ITP patients (2 males, 9 females; median age 46.3 years, 28.6-67.8) were treated with Rituximab (375 mg/m²/weekly for four doses). Median time between diagnosis and start of Rituximab was 4.1 years (0.2-33.1 months). All patients had already received at least two lines of therapy (median 3; 2-6); prednisone, pulsed high-dose dexamethasone, azathioprine, immunoglobulins, interferon or splenectomy. Therapy consisted of no drug treatment in 9 patients (5.6%), intravenous pulses of steroids in 6 patients (33%), cyclophosphamide in 6 patients (33%), and cyclosporine in 1 patient (5.6%). After completing therapy, patients were evaluated for platelet count after 1 and 3 months, and thereafter every 3 months until relapse or start of a different treatment. Peripheral blood B lymphocytes were evaluated by flow-cytometry as CD19+ cells before treatment, 1 and 3 months after stopping therapy, and then every 3 months up to recovery. Results. One month after Rituximab therapy, 5 responses (1 CR, 3 PR, 1 MR; 45%) and 3 NR (15%) were observed. Two relapses occurred 5 and 18 months after response. The median follow-up of all treated patients was 8.7 months (1.8-31.1), while the median follow-up of all responsive patients is 13.7 months (2.6-18.7). Before starting therapy, 9/11 cases were evaluable for flow-cytometry studies. The median baseline value of peripheral blood CD19+ cells was 128×10⁹/L (59-371). One month after completing therapy, 6/8 evaluable cases showed absence of CD19+ cells and 2/8 showed a count of 9 and 4.4×10⁹/L CD19+ cells, respectively. At the last available control (median follow-up of 11 months; 1-28), 8/9 patients had still not recovered the baseline CD19+ cell count (median value: 6×10⁹/L; 0-295). The following side effects were observed: 3 cases of papulosquamous dermatis, 1 case of fever and 1 case of fever and dermatitis. **Conclusions.** Five/11 (45%) ITP patients had an early response to Rituximab (1 CR, 3 PR, 1 MR), that persisted in 3 cases. No late responses were observed. The response was independent from the post-therapy CD19+ cell count (median value: 6×10⁹/L; 0-295). The median CD19+ cell count of 1000-15000/mm³ at diagnosis ranged from 1000 to 157000/mm³ (median 80900 - mean 78000). The clinical classification at diagnosis was: 25% (95% C.I.: 20.0 - 30.4) severe - PLT under 5000/mm³ - 36% (C.I.: 30.3 - 41.9) moderate - 51000-100000/mm³ - 39% (C.I.: 33.1 - 44.8) mild - 101000-150000/mm³; at last follow up these percentages changed to: 10% severe - 29% moderate - 33% mild and 28% normal (>150000/mm³). Pseudotrabomucopenias resulted in 31 patients (11% - C.I.: 7.6-15.5; 9 men (28.6%) and 22 women (71.4%); p=0.12); 1 patient (3.2%) was successively diagnosed with cutaneous bleeds with no clinical presentation of only thrombocytopenia. **Conclusions.** The overall probability of hemorrhagic events in these patients is 10% (95% C.I.: 7-15) and the probability of severe hemorrhagic event of 0.4% (95% C.I.:0.01-2); none fatal event has occurred (95% C.I.:0.1-1). The outcome of isolated thrombocytopenias appear very favourable and therapy can be performed in a minority of cases (17%).

**0934**

**IDIOPATHIC THROMBOCYTOPENIC PURPURA IN CHILDHOOD: REVIEW OF 160 CASES**

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Idiopathic thrombocytopenic purpura (ITP) is one of the most common acquired bleeding disorders in childhood. Usually it is a benign self-limited disease that occurs in previously healthy children. The purpose of the present study was to review the clinical course and management of all children with ITP admitted over eight-year period to the Pediatric Clinic in Skopje. One hundred and sixty cases were identified indicating an incidence of 4/100000 children under 15 years. The sex ratio (female/male) was 1.59; 1.1; (1.4) and the median value of the age between 2-10 years, 36 (22,5%) under 2 years and 30 (18,5%) older than 10 years. 88 (55%) presented with cutaneous signs only, and 65 (40,6%) had mild mucous membrane bleeding. Just 12 (7%) had severe bleeding symptoms: 8 (4,8%) nosebleeds requiring nasal packing, 3 (1,8%) increased menstrual bleeding and 1 (6,2%) macrohematuria. No patient suffered an ICH or severe bleeding requiring transfusion. The mean platelet count on admission was 21,4×10⁹/L, lowest count 3×10⁹/L. Bone marrow aspiration was performed almost in all cases. Initial management consisted of no drug treatment in 9 patients (5,6%), intravenous immunoglobulins (IVIG) in 17 (10,6%) and glucocorticosteroids (GS) in 134 (83,5%). IVIG were used just in infants as they were expensive form of treatment. All patients improved regardless of the management strategy used. The mean length of stay in hospital in the period between 1999-2001 was 14,5 days, and between 2002-2005 7,9 days. We consider it could be reduced more and majority of patients, particularly those with cutaneous bleedings only, could be treated as outpatients. Conclusions. Children presenting with ITP have a low incidence of bleeding complications and a good response to standard treatment with oral prednisone. Many of these patients can be managed as outpatients.
**935**

**AUTOIMMUNE THROMBOCYTOPENIA: CLINICAL AND HEMATOLOGICAL OUTCOME OF PATIENTS AFTER DANAZOL ADMINISTRATION AS FIRST LINE OR REFRACTORY DISEASE TREATMENT**


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**Introduction.** First line treatment of Autoimmune thrombocytopenia (AT) consists of corticosteroids and intravenous immunoglobulin. In refractory cases, splenectomy is indicated. In older patients or in those with a worse performance status, long term corticosteroid administration and splenectomy may be harmful and alternative treatments are under evaluation. Danazol is an androgen with mild, rare and reversible adverse events, which plays a role in AT therapy. **Aim of the study.** The aim of our study was to estimate the effectiveness and safety of danazol treatment in newly diagnosed or relapsed patients with AT. **Patients and Methods.** 35 patients (25 male/12 female) suffering from AT (ATP, 4-MDS/ITP, 1 low grade-NHL/ITP) were studied from 2002 to date. Median age was 65 years (23-92 years). 21 patients were >60yo and 14 patients were <60 years and relapsed. 4:MDS/ITP, 1:low grade-NHL/ITP were studied from 2002 to date. The overall response rate in patients treated with danazol alone (23/35) was 56,5% (CR 39,1%, PR 17,4%).

<table>
<thead>
<tr>
<th>Initial treatment</th>
<th>Treatment options</th>
<th>CR</th>
<th>PR</th>
<th>MR</th>
<th>NR</th>
<th>NA</th>
<th>SUM</th>
</tr>
</thead>
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<tr>
<td>Initial IR**</td>
<td>Danazol alone</td>
<td>49</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>7</td>
</tr>
<tr>
<td>continued with danazol alone</td>
<td>Interim response to danazol→relapse→IR→continue with danazol alone</td>
<td>22</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>&gt;60 years, first diagnosed</td>
<td>Danazol alone</td>
<td>22</td>
<td>2</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Interim response to danazol→relapse→IR→continue with danazol alone</td>
<td>13</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Danazol alone</td>
<td>72</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Interim response to danazol→relapse→IR→continue with danazol alone</td>
<td>31</td>
<td>1</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>&lt;60 years, danazol alone relapsed*</td>
<td>Danazol alone</td>
<td>19</td>
<td>13</td>
<td>2</td>
<td>1</td>
<td>35</td>
<td></td>
</tr>
</tbody>
</table>

**Table 1.**

**Discussion.** In patients over 60yo treated with primary IR and continued with danazol alone (12/35) the response rate was 58,3% (CR 38,5%, PR 20,8%). In patients with interim response to danazol→either alone or after initial IR administration- 40% (14/35) relapsed during follow up. These patients were treated with a second IR and returned to danazol monotherapy. 86% of them achieved second response to danazol (CR 43%, PR 43%) (Table 1). Co-administration of low dose methylprednisolone induced the response rate to danazol without causing standard corticosteroid adverse events. Average duration of response to danazol was 19 months, median was 7 months. The median time to response to danazol was 1 month. 97% (34/35) of patients showed clinical response and 31,4% (11/35) had adverse events due to danazol treatment: 9/35 had elevated liver enzymes (2/9 drug cessation due to severe reversible transaminasemia), 2/35 had mild renal function impairment. Discussion. Danazol is a safe and cheap treatment for autoimmune thrombocytopenia, as second line therapy in young relapsing patients, or as first line treatment in older patients, because long-term corticosteroid treatment is avoided with this schedule.

**936**

**DESCRIPTIVE EPIDEMIOLOGY OF IMMUNE THROMBOCYTOPENIC PURPURA IN THREE EUROPEAN COUNTRIES**

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**Backgrounds.** The incidence and prevalence of immune thrombocytopenic purpura (ITP) has not been well characterized to date. **Aims.** To characterize ITP incidence and prevalence in three European countries overall and according to sex. Also, to determine whether ITP incidence and prevalence rates are increasing. **Methods.** Incident and prevalent cases were identified from databases from three countries: United Kingdom (General Practitioners Research Database (GPRD) 1990 through 2000), Germany (IMS Disease Analyzer Medipius 1994 through 2008), and Netherlands (PHARMO database 1991-2005). GPRD and IMS include general practice physicians chosen such that their patients are representative of their respective countries. IMS also includes specialists. PHARMO contains hospitalization data from the National Medical Registry covering all hospital admissions in the Netherlands. Dutch population counts were obtained from Statistics Netherlands. ITP diagnoses were identified using the relevant coding system/codes for each database: ICD-9 287.3 (PHARMO), ICD-10 D693 (IMS Disease Analyser Germany), and Read and OXMIS codes corresponding to an ITP diagnosis (GPRD). Incidence rates include only first time diagnoses, whereas prevalence rates include new and recurrent diagnoses. **Results.** The average annual incidence rate in the UK was 3.0 per 100,000 person years (95% confidence interval (CI) 2.7 to 3.3). Rates were fairly stable over time, ranging from 1.0 per 100,000 person years in 1998 to 3.7 per 100,000 in 1991. Average incidence, unadjusted for age, was 3.4 per 100,000 person years for women and 2.5 per 100,000 for men. Prevalence rates ranged from a low of 2.1 per 100,000 person years in 1990 and 2000 to a high of 8.1 per 100,000 in 1997. For Germany, average annual incidence was 2.7 per 100,000 person years (95% CI 1.7 to 4.1), ranging from 1.6 per 100,000 in 1996 to 5.1 per 100,000 in 1994. Incidence rates were comparable for German men and women. Prevalence ranged from 2.6 per 100,000 person years in 1999 to 7.5 per 100,000 in 1994. In the Netherlands, average annual incidence was 1.9 per 100,000 person years (95% CI 1.8, 2.0) varying little from 1991 through 2003 (1.7 to 2.1 per 100,000 person years). Average incidence was slightly higher for women than for men (2.2 per 100,000 and 1.8 per 100,000 person years, respectively). Annual prevalence ranged from 1.9 per 100,000 to 2.4 per 100,000 person years. Summary/Conclusions. ITP incidence and prevalence rates were less than 5 and 10 per 100,000 person years, respectively, in three major European countries. Incidence rates were higher for women than men in the UK and the Netherlands, but not in Germany. Rates did not increase during the period 1990 through 2003. These analyses of general practice and national medical databases provide a robust picture of recent ITP incidence and prevalence with a degree of precision lacking in previous evaluations of this relatively rare condition.
THE SALVAGE TREATMENT WITH CYCLOSPORIN A IN IDIOPATHIC THROMBOCYTOPENIC PURPURA

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Backgrounds. Physicians face therapeutic dilemmas when patients become resistant to known treatment in life-threatening conditions. A review of the literature shows a lack of comprehensive information on the clinical use of Cyclosporin A in the treatment of idiopathic thrombocytopenic purpura (ITP). Aims. To verify the usefulness of Cyclosporin A therapy in refractory ITP. Method. Study was carried out on long-term treatment (median 36 months) with Cyclosporin A (3 mg/Kg/day) in 14 selected adult patients with chronic, severe ITP resistant to all the usual therapies. Results. A median follow-up of 26.2 months shows that Cyclosporin A treatment obtained an improvement in 10 out of 14 patients (71%); 9 patients (64%) achieved a complete response and one (7%) a partial response. Four patients (28%) were unresponsive. In 5 patients (50%) the discontinuation of Cyclosporin A was successful, maintaining the remission over time, without further therapy. In the remaining responsive patients, the remission was dependent on continued drug. The Cyclosporin A intolerance was slight in spite of long-term treatment (median 36 months) with Cyclosporin A (3 mg/Kg/day) in 14 selected adult patients with chronic, severe ITP resistant to all the usual therapies. 

Cyclosporin A therapy obtained an improvement in 10 out of 14 patients (71%); 9 patients (64%) achieved a complete response and one (7%) a partial response. Four patients (28%) were unresponsive. In 5 patients (50%) the discontinuation of Cyclosporin A was successful, maintaining the remission over time, without further therapy. In the remaining responsive patients, the remission was dependent on continued drug. The Cyclosporin A intolerance was slight in spite of long-term treatment (median 36 months) with Cyclosporin A (3 mg/Kg/day) in 14 selected adult patients with chronic, severe ITP resistant to all the usual therapies.

Because the potential role in second neoplasia appearance and the well known teratogenic role of this immunosuppressor, cyclosporin A will be done only in resistant ITP cases (dramatic clinical cases).
and the rate of apoptosis represent laboratory markers that may be related to the progression of pathologic clone in patients with myelodysplastic syndrome (MDS). In this study we investigated these markers in patients with different subtypes of MDS. Methods. X-chromosome inactivation pattern clonality assay based on PCR amplification of polymorphic short tandem repeats of the human androgen receptor (HUMARA) gene was performed in granulocytes, CD14+ and CD34+ cell subpopulations isolated from bone marrow and peripheral blood of 58 females with primary MDS and 20 healthy controls. The results were compared with measurement of the telomere length by Terminal Repeat Fragment (TRF) method and with apoptotic rate of CD34+ and GlyA+ subpopulations assessed by flow cytometry (Annexin V and TUNEL methods). Results. In 19 patients with advanced MDS (RAEB, RAEB-T, CMML, anticipation of the FAB classification in pre-B-precursor acute lymphoblastic leukemia) CD14+ cell subpopulations (allele ratio ≥0.91) were present in bone marrow and peripheral blood of 74% and 87% of patients, respectively. Shortened telomere length (TRF <7.5 kb) and low rate of apoptosis of CD34+ bone marrow cell subpopulation were present in all patients with advanced MDS. In patients with early MDS, clonal patterns of hematopoiesis were present only in 2 out of 17 patients (12%) with RA, RARS or 5q- syndrome according to the WHO classification. On the other hand, clonal granulocyte or CD14+ cell subpopulations were present in bone marrow or peripheral blood of 20 out of 22 patients (90%) with RCDM, according to WHO criteria. In accordance with these results, patients with early MDS and non-clonal granulocyte cell subpopulations exhibited low apoptotic rate of CD34+ bone marrow cells (5-12%). On the contrary, 80% of patients with non-clonal cells had increased apoptotic rate of CD34+ cells (30-80%). Reduced telomere length was found in 71% patients with clonal cell subpopulations vs. 45% in non-clonal cells. Median survival of patients with early MDS and clonal cells was 62.5 months vs. 47.8 months in those with non-clonal cells (p=0.05) and 65.7 months in RA patients vs. 50.0 months in RCDM patients (p=0.05). Conclusions. The results confirm our preliminary observations suggesting that RCDM represents a separate clinical and laboratory entity with adverse prognosis which is different from early MDS. Clinical characteristics and laboratory markers that may be related to the progression of pathologic clone in patients with myelodysplastic syndrome (MDS) suffer from recurrent bacterial infections as a result of differentiation defects of the neutrophil lineage. While limited number of genetic defects of MDS progenitor cells has been described, the defective intercellular signal transduction pathways modulating these developmental defects remain undefined. Mitogen-activated protein (MAP) kinase cascades play a key role in regulating a plethora of cellular processes. They typically are organized in a three-kinase architecture consisting of a MAPK, MAPK activator (MKK or MAPK kinase), and a MKK activator (MAPK kinase kinase). The p38 MAPK pathway mediates a wide variety of cellular processes in response to extracellular stimuli such as UV light, osmotic shock, inflammatory cytokines and growth factors and it has been shown that MKK3 and MKK6 are the main MKKs activating p38. Although p38 has been demonstrated to regulate differentiation in several cell types, its role in regulating neutrophil development in both normal as well as in defective MDS granulopoiesis remains to be investigated. Aims. The aim of this study was to investigate the role of the p38 MAPK signalling module in neutrophil differentiation and to determine whether p38 MAPK signalling may play a role in aberrant neutrophil development in MDS. Mononuclear cells were isolated from umbilical cord blood using a ficoll-paque solution and MACS immunomagnetic cell separation was used to isolate CD34+ cells. Cells were cultured in IMDM supplemented with 9% serum and differentiation towards neutrophils was induced upon stimulation with GM-CSF-G-CSF. Nuclear apoptosis was assessed by light microscopy. Processing of caspases, cytochrome-c release into the cytosol, cleavage of PARP, expression and cleavage of Bcl-2 family proteins were analyzed by Western blot. Loss of mitochondrial membrane potential was estimated by FACS analysis using the fluorescent dye TMRE. Mitochondrial involvement clonality assay based on PCR amplification of polymorphic short tandem repeats of the human androgen receptor (HUMARA) gene was performed in granulocytes, CD14+ and CD34+ cell subpopulations (allele ratio ≥0.91) were present in bone marrow and peripheral blood of 74% and 87% of patients, respectively. Shortened telomere length (TRF <7.5 kb) and low rate of apoptosis of CD34+ bone marrow cell subpopulation were present in all patients with advanced MDS. In patients with early MDS, clonal patterns of hematopoiesis were present only in 2 out of 17 patients (12%) with RA, RARS or 5q- syndrome according to the WHO classification. On the other hand, clonal granulocyte or CD14+ cell subpopulations were present in bone marrow or peripheral blood of 20 out of 22 patients (90%) with RCDM, according to WHO criteria. In accordance with these results, patients with early MDS and non-clonal granulocyte cell subpopulations exhibited low apoptotic rate of CD34+ bone marrow cells (5-12%). On the contrary, 80% of patients with non-clonal cells had increased apoptotic rate of CD34+ cells (30-80%). Reduced telomere length was found in 71% patients with clonal cell subpopulations vs. 45% in non-clonal cells. Median survival of patients with early MDS and clonal cells was 62.5 months vs. 47.8 months in those with non-clonal cells (p=0.05) and 65.7 months in RA patients vs. 50.0 months in RCDM patients (p=0.05). Conclusions. The results confirm our preliminary observations suggesting that RCDM represents a separate clinical and laboratory entity with adverse prognosis which is different from early MDS.
seen by different health care providers. Aims. We gathered data on patient characteristics and treatment of 217 new MDS patients seen in our medical center during the year 2005. Methods. All MDS patients treated either in the hematology outpatient clinic or on the wards were documented. A diagnosis of MDS was made according to the standards of the German MDS Registry in Düsseldorf, including central morphology. Patients were followed for the incidence of complications, disease progression, and therapeutic intervention during the year 2005. Results. In 2005, a total of 217 patients were seen at our institution. In 90 patients (41%) the diagnosis of MDS was made during that year, either before or after referral. 93% had primary MDS, 7% were diagnosed as treatment-related MDS. The distribution among MDS types was: 10 RA, 4 RARS, 64 RCMD, 26 RCMD-RS, 26 RAEB I, 33 RAEB II, 25 CMML, 6 patients with 5q- syndrome, and 23 patients with RAEB-T. A karyotype analysis was available in 82% of patients (n=179). 100 patients (56%) pre-sented with a normal karyotype. According to the International Prognostic Scoring System (IPSS), 25% of the patients belonged to the low-risk, 36% to the Intermediate-1, 24% to the Intermediate 2, and 15% to the high-risk group. A 5q- anomaly. Either as sole abnormality or as part of a more extensive derangement, was found in 17 patients. There were 121 males and 96 females. 128 (59%) patients were treated in our outpatient clinic only, with a median number of 4 consultations with the doctor (1-67). 89 (41%) pa-tients were admitted to the hospital, 60 of whom (51%) were treated on the ward as well as in the outpatient clinic. Reasons for hospitalization were disease complications like infections, hemorrhages, and bad general condition in 55%. In 45% of cas-es, patients were admitted for intensive chemotherapy, allogeneic stem cell transplantation, or any kind of treatment that requires inpatient care, including the institution of treatment. The median number of hospitalizations per patient was 1 (1-6). 29 patients (13%) died during the year 2005, 59 patients (27%) showed progression to AML. 81% of patients received at least one unit of packed red cells. Summary: With regard to MDS sub-type distribution, patients seen in our institution did not differ much from the MDS population as a whole. Still, a referral bias is present, reflected by a large proportion of patients requiring inpatient care, either for management of MDS-related complications or intensive treatment of the underlying bone marrow disease.

0943

ACQUIRED α-THALASSEMIA IN MDS (AT-MDS): RARE MUTATIONS DETECTED IN TWO FEMALES

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Background. In contrast to the classical thalassemias, two distinct thalassemias were recently described in which the molecular defect does not reside in the globin genes but in a transcriptional activator of α-globin genes. This protein, dubbed ATRX, is mutated in the rare inherited dis-ease of α-thalassemia (AT) with mental retardation (ATR-X syndrome) whose affected individuals show a mild form of AT. In addition, and independent of the ATR-X syndrome, there have been approximately 100 case reports worldwide of the association of an acquired form of AT with hematological neoplasms, the large majority of those cases being MDS (ATMDS). The clinical characteristics of such patients encompass the typical features of the underlying hematological disorder plus microcytic anemia. The latter is due to massively reduced α-globin gene tran-scription resulting in excess hemoglobin H (HbH), as revealed by supravital stain-ing of peripheral blood smears. Molecular mechanisms by which muta-tions cause acquired α-thalassemia probably include epigenetic alter-tations of DNA methylation and chromatin structure. The remarkable thrombocytopenia and erythrocytosis, respectively, in our 2 pts are at least suggestive of other phenotypic abnormalities possibly associated with the acquired ATRX genotypes on the MDS background.

0944

SERIAL DETERMINATION OF FLT3 MUTATIONS IN MDS PATIENTS AT DIAGNOSIS, FOLLOW UP, OR AML TRANSFORMATION: FLT3 ITD/ASPS33 MUTATIONS OCCURRENCE AND THEIR PROGNOSTIC SIGNIFICANCE


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Background. The genetic/molecular alterations that lead to MDS are not fully elucidated. MDS can be considered as pre-leukemia but the precise genetic/molecular events occurring during transition to AML are unknown. Aim. The aim of this study was a) to investigate the incidence of FLT3 mutations (ITD/Asp335) in MDS patients at the time of MDS diagnosis and during disease evolution, b) to analyze if the presence of FLT3 mutations correlates to AML transformation and c) to investigate the prognostic significance of FLT3 mutations in MDS patients. Methods. Genomic DNA was extracted from bone marrow aspirate smears from 97 patients with MDS (RAEB-t and therapy-related MDS were excluded). All patients had bone marrow smears at presentation and at several time points during their follow up (2-10 samples per patient). Patient DNA was amplified by PCR with specific primers for the detection of FLT3 internal tandem duplication (ITD). ITD positive samples (PCR band(s)>329bp) were cloned and sequenced. Asp355 point mutations in exon 20 of the FLT3 gene were detected with PCR followed by diges-tion with EcoRV of the 195 bp PCR product. Non fully digested prod-ucts (mutated) were cloned and sequenced (10 plasmids/patient) to verif-y the existence of Asp355 mutation. Fisher’s exact test and unpaired t-test were used for statistical analysis. Survival curves comparison was done by the log-rank test. For all analyses the p-values <0.05 were con-sidered statistically significant. Results. Three of the 97 patients had FLT3 mutations at presentation: one patient with both ITD and Asp335 (RAEB-BM blasts 16%), one patient with ITD only (RAEB) and one patient with Asp335 only (RAEB). Forty two patients progressed to AML including the three patients that carried FLT3 mutations at MDS diagno-sis. The total incidence of FLT3 mutations at the time of AML progres-sion was 14.3% (6 out of 42), with 3 additional patients acquiring FLT3 mutations at AML progression. In these 3 latter patients, FLT3 mutations were detectable in bone marrow samples 4-6 weeks before overt leukemic transformation. All identified FLT3 mutations were in frame as shown by sequence analysis and suggest a gain of func-tional mutational event. Patients with FLT3 mutations had 4.5 times higher risk of transformation to AML compared to patients without muta-tions (Cox’s model application). Survival curves comparison by long-rank test showed a statistical significant difference between MDS patients with FLT3 mutations compared with those without mutations (p=0.0001), as well as between transformed MDS patients with FLT3 mutations compared with transformed MDS patients without muta-tions (p=0.01). Two extra patients acquired FLT3 mutations 12 and 32 months respectively after MDS diagnosis; both these patients died 2 and 6 months respectively, after FLT3 mutation detection, from infection, before evolution to AML. Conclusion. Our study shows that FLT3 muta-tions seem to be the critical additional genetic event that transforms a minority of MDS to AML; these mutations can be detected before trans-formation to AML and effective FLT3 inhibitors, when available, might be a potent therapeutic modality for these patients.
INCIDENCE OF MDS WITH 5Q- KARYOTYPE ANOMALIES

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Background. In a previous study (Germing et al., Haematologica, 2004) we found an overall incidence of myelodysplastic syndromes (MDS) of ~5/100,000 per year in the town district of Düsseldorf. The incidence figures were strongly age-related, with a significant rise after the age of 60, particularly in males. We calculated that approximately 4100 new cases of MDS are diagnosed in Germany each year. In individuals below the age of 40 years, the incidence of MDS is only ~0.4/100,000 per year. Because of the 'greying of the population' in developed countries, study of peripheral blood progenitor cell growth from different populations is desirable as a diagnostic tool to ascertain or to rule out diagnosis of early MDS.

METHODS. Bone marrow (BM) and peripheral blood (PB) was collected from patients with unknown origin and stained with MGG to look for colonies growth (number and morphology). Results. Median number of colonies (CFU-GM and BFU-E) for 105 MNC was similar for T (n=11) and C (n=27), but lower for MDS patients (n=5) (p<0.0004). Median clonogenic efficiency of CFU-GM was 3 times lower for BM-GM and 14 times lower for BFU-E in comparison to non malignant secondary cytopenias (p<0.001). Morphologic analysis of colonies from collagen gels allowed estimating cellular degeneration: the ratio viable colonies/all colonies (v/a), for both CFU-GM and BFU-E, was always >0.60 in T and C groups, whereas it was always <0.50 in MDS patients (p<0.003). Among the 13 patients displaying cytopenias of uncertain diagnosis, three had a CFU-GM and/or BFU-E v/a ratio<0.58, and 10 a v/a ratio >0.60. The three patients with an abnormal v/a ratio evolved to a MDS (RAEB or RCMC) within 2 years following cell culture. Nine patients with a normal v/a ratio recovered a normal cell count within 6 months (n=6) or developed progressive kidney failure (n=5). One patient with normal v/a ratio remained unclassified after 9 months of follow up. Conclusions. Whatever the MDS subtype, cell culture demonstrated that abnormal progenitors were consistently found within peripheral blood, which demonstrated limited in vitro growth and a high degeneration rate (apoptosis%). As a high degeneration rate of progenitors was not observed in non malignant disorders, study of peripheral blood progenitors in myelodysplastic patients of unknown origin is proposed as a diagnostic tool to ascertain or to rule out diagnosis of early MDS.

ALTERNATIONS IN THE NATURAL KILLER CELL RECEPTOR REPERTOIRE IN MYELODYSPLASTIC SYNDROMES

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Background. Myelodysplastic syndromes (MDS) constitute a group of clonal stem cell disorders characterized by ineffective hematopoiesis and pancytopenia. In one third of the MDS patients the disease progresses to acute myeloid leukaemia. The only curative treatment for MDS is allogeneic stem cell transplantation (SCT). Several studies have shown that natural killer (NK) cells play an important role in the outcome of SCT in acute myeloid leukaemia patients. These results suggest that NK cell mobilization constitutes an important therapeutic tool in the treatment of hematological diseases such as MDS. So far, the role of NK cells in the pathogenesis of MDS is poorly understood. Aim: The aim of this study was to investigate the NK cell receptor repertoire in patients with MDS. Methods. Bone marrow (BM) and peripheral blood (PB) was collected from patients with MDS and NK cells were analyzed for their receptor repertoire using multi-color flow cytometry. Results. MDS patients displayed severe alterations in their NK cell receptor repertoire with decreased expression of several activating NK receptors, including DNAM-1, 284, NKG2D, and CD16. These alterations were confined to BM-derived NK cells and did not affect NK cells in PB. One patient had abnormally high levels of CD56bright NK cells displaying a reversed ratio between CD56bright and CD56dim NK cells with 75% and 50% regulatory CD56dim NK cells in BM and PB, respectively. Conclusions. Our preliminary results show that MDS patients display several phenotypic aberrations in their NK cell repertoire. This may have functional consequences and influence patient prognosis and response to immunomodulatory treatments for MDS. Uncovering a role for NK cells in the recognition of MDS tumor cells may set the stage for future NK cell-based immune therapies against MDS.

MYELOID ANTIGEN EXPRESSION ON CD34 POSITIVE BLASTS IN MYELODYSPLASTIC SYNDROMES

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Background. The myelodysplastic syndromes (MDS) constitute a heterogeneous group of clonal hematopoietic stem cell disorders. They are characterized by abnormal bone marrow (BM) differentiation, peripheral blood cytopenias and a risk of transformation into acute myeloid leukemia (AML). The diagnosis of MDS depends on morphological criteria and cytogenetics and is sometimes difficult to make and subjective. Aim: In this study we evaluated the potential of immunophenotyping CD34+ hematopoietic precursors for the diagnosis and classification of myelodysplastic syndromes. Methods. Bone marrow samples of patients with different forms of MDS (51 samples), of healthy controls (15 samples), of patients with cytopenia not due to MDS (57 samples) and of patients with AML (25 samples) were examined. MDS (36%), chromosomal analyses were performed at the time of diagnosis. Patients were karyotyped significantly younger (median: 64 years) than the MDS patient population as a whole (p=0.0065). Among those with a karyotype available, 180 patients (17%) had a 5q- anomaly, either as a single aberration (n=114) or together with one more chromosomal abnormality (n=12), or as part of a complex karyotype (n=53). This implies an incidence of MDS with 5q- anomalies of about 0.85/100,000 per year, equivalent to 70 new cases per year in Germany. Among patients with a 5q- abnormality in our database, we identified only 21 females younger than 50 yrs and 7 females younger than 40. For males, the figures were similar. To summarize, MDS is one of the most frequent haematological disorders, particularly in the elderly. Patients with karyotype anomalies involving 5q- represent about 17% of MDS patients with an estimated incidence of about 0.85/100,000 per year. Such patients are rare among individuals less than 50 years old. Because of the ‘greying of the population’ in developed countries, the number of all MDS patients, including those with a 5q- anomaly, is expected to rise.
and AML samples were classified according to the WHO criteria. The expression of CD19, CD10, CD133, CD15, CD83, CD117 and CD45 antigens was detected on the CD34+ cells by flow cytometry. Statistical analysis was done with a Mann-Whitney test. Results. The number and immunophenotype of the CD34+ cells in BM of disease controls was similar to that in normal bone marrow. Only the number of CD34+ CD117+3+ cells was low. A higher number of CD34+ cells was found in MDS and AML. This number correlated with the percentage of blasts found by cytomorphology. The increase of the CD34+ cell number was accompanied by an increase of the myeloid precursors (CD34+ CD117+3+) and a decrease of the B cell precursors (CD34+ CD19+). CD117 appeared to be the best marker for myeloid precursors, followed by CD13, especially when the number of blasts was high. A wide range of CD34+ CD133+ and of CD34+ CD133+ positive cells was found in all types of samples. CD133 expression was increased in MDS samples with excess of blasts and in AML. No statistical difference was found between the different groups for the CD34 expression. The myeloid antigen expression on CD34+ cells in refractory anemia (RA), refractory anemia with ringed sideroblasts (RARS) and refractory cytopenia with multilineage dysplasia (RCMD) was comparable, although a low positivity for CD13 was found in RARS patients. In MDS with excess of blasts, no statistical differences were found between the myeloid antigen expression on the CD34+ cells of the two subtypes (RAEB-1 and RAEB-2). The phenotype of myeloid precursors in AML patients (CD34+ CD13+, previously RAEB-0) was comparable to that found in AML with multilineage dysplasia and other AML patients. Conclusion. MDS is characterized by a variable number of CD34+ cells in the bone marrow. This number correlated with the percentage of blasts found by cytomorphology. An increase of the number of CD34+ is accompanied by an increase of the myeloid precursors and a decrease of the B lymphoid precursors. In MDS samples with less than 5% blasts the myeloid antigen expression on the blasts was comparable to that in disease controls. In MDS samples with an excess of blasts the phenotype was closer to that of AML.

9049
LOW-RISK MYELODYSPLASTIC SYNDROMES FROM PIEMONTE MDS REGISTRY: A COMPARATIVE REVIEW OF BONE MARROW ASPIRATE SMEARS AND BONE MARROW BIOPSY SPECIMENS
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Aims. To assess the contribute of bone marrow aspirate (BMA) and bone marrow biopsy (BMB) on diagnosis and prognosis of low-risk MDS. Patients and methods. We reviewed 82 cases of MDS with low marrow blasts (≤5%), consecutively admitted in five hospital of Piedmont between 1998 and 2004. All patients were studied on admission, with full blood count, cytogenetics, BMA and BMB. Pt's notes were recorded in the archives of Piedmont MDS Registry. Prerequisites for evaluation of diagnostic features in MDS included the viability of May Grumwald-Giemsa well stained BMA smears and BMB specimens. BMA were examined for dyserythropoiesis (DE), dysgranulopoiesis (DG) and dysmegakaryopoiesis (DM), as defined by WHO criteria, the percentage of blasts and ringed sideroblasts by a panel of well-trained hematologists. The same was done by two expert pathologists. Inter-observer reproducibility between hematologists and pathologists was estimated. Median survival were calculated by Kaplan-Meyer analysis. Results. Median follow up was 27.8 months (0-151). The inter-observer agreement for dysplasia was: good for DE and low for DG, DM and multilineage dysplasia (MuLD). Patients with definite clinical entries such as 5q-syndrome (2 cases), del(5q) and del(5q)/AF5q (3 cases with thrombocytocytopenia or neutropenia) and RAEB (8 cases presenting on BMB a percentage of blasts over 5%) were excluded from analysis. 19 cases presented anemia (group A) and 48 pancytopenia (group B). In 14 cases of group A a full concordance was recorded: 4 showed DE and 10 MuLD. By contrast in 5 cases of group B, CD34+ was recorded in BMA while in BMB only DE was apparent. In group B a full concordance was recorded in 34 cases; 32 showed multilineage dysplasia and 2 only DE. By contrast in 4 cases showing MuLD on BMA, BMB showed DE and 5 cases showing DE on BMA, presented MuLD on BMB. For group A median survival was not reached while group B had a median survival of 40 months (Log rank test=7.2 p<0.05). Median survival of DE and MuLD were not reached (after 150 months) and 40 months, respectively (p<0.05). The same analysis was not statistically significant on aspirate. Conclusions BMA allowed a reclassification in RAEB in about 10% (8/82) of the cases showing a percentage of blasts at the aspirate lower at 5%. BMA has been able to identify more accurately pure RA with better prognosis. BMB should be reserved to patients with proven diagnosis of MDS while BMA should be used as first-line, baseline procedure in patients with suspected MDS.

9050
OCCURRENCE OF THE JAK2 V617F MUTATION IN PATIENTS WITH REFRACTORY ANEMIA WITH RINGED SIDEROBLASTS ASSOCIATED WITH THROMBOCYTOPENIA (RARS-T)
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Backgrounds. The WHO classification establishes a new category, the Myelodysplastic/Mypeloproliferative diseases (MDS/MPD). This category includes myeloid disorders that have both dysplastic and myeloproliferative features. MDS/MPD, U-refractory anemia with ringed sideroblasts associated with marked thrombocytosis (RARS-T) is incorporated in this category as a provisional entity. The clinical and morphological features consist of the myelodysplastic syndrome, refractory anemia with ringed sideroblasts (RARS) but with a marked thrombocytosis (>600x10^9/L). The megakaryocytes are enlarged in size. Essential Thrombocytemia (ET) is a Chronic Myeloproliferative Diseases (MPD). A single point mutation of JAK2 (Val617Phe) has been detected in half the patients with ET. Aims. The presence of the JAK2 mutation was assessed in a cohort of patients with RARS, including 3 cases with RARS-T. Methods. We obtained DNA from blood samples from 3 patients with RARS-T. These samples were analyzed using the allele-specific PCR methodology described by Baxter et al.1 DNA samples from 16 RARS and from one ET were also studied. Results. In the three cases with RARS-T, the V617F mutation of the JAK2 gene was detected, but none of the other cases with RARS showed the mutation. Interestingly, endogenous erythroid colony formation in vitro was negative in two of them. Bone marrow exams showed hypercellularity with prominent megakaryocytic proliferation, enlarged in size. None of them showed the typical small-sized megakaryocytes of the 5q- syndrome. After a long follow-up (15 years) one case evolved to myelofibrosis. In the ET group, 13 out of 21 cases showed the JAK2 mutation. Conclusion. RARS-T appears to be the coexistence of two disorders, with erythropoiesis showing the characteristics of the RARS and megakaryocytes those of ET. Further data from other groups are necessary to confirm the prevalence of the JAK2 mutation in RARS-T.

References

9051
WT1 IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES: A USEFUL MOLECULAR MARKER FOR RISK ASSESSMENT
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Backgrounds. Myelodysplastic syndromes (MDS) are clonal hematopoietic stem-cell disorders characterized by ineffective dysplastic hematopoiesis involving one or more cell lineages and characterized by peripheral-blood cytopenia and a high risk of progression to acute myeloid leukemia (AML). According to WHO classification, MDS can be classified in these following groups: refractory anemia (RA), RA with ringed sideroblasts (RARS), RA with excess of blasts type I and II (RAEB I and II), refractory cytopenia with multilineage dysplasia (RC+Dys), del(5q) syndrome, and MDS unclassifiable (MDS unclass). The Wilms’ tumor gene (WT1) is a tumor suppressor gene coding for a zinc-finger transcription factor located on chromosome 11p13, which was originally identified for its involvement in the pathogenesis of the Wilms’ tumor. In normal peripheral blood (PB) and bone marrow (BM), WT1 expression is reported to be low and sometimes undetectable even by RT-PCR. By contrast, WT1 is highly expressed in most acute leukemias, and its level of expression is associated with the presence, persistence, or reappearance of leukemic hematopoiesis. Aims. WT1 gene expression could represent a useful marker in MDS to establish prognosis and progression of disease. Methods. BM samples from 36 MDS patients (16 RA, 7 RAEB I, 4 RAEB II, 4 RARS, 8 deletion of 5q, 2 MDS unclass) were tested for WT1 expression at diagnosis and after 6 months. WT1 gene expression
was evaluated by methods of real-time quantitative PCR (RQ-PCR). Results. At diagnosis, 218M samples (10 RA, 6 RAEB I, 4 RAEB II, 1 RARS, 1 MDS unclass) expressed WT1 transcript amounts greater than the ranges level. The degree of WT1 expression was highly correlated with the type of MDS, was much higher in RAEB I and II compared with RA, and other types, and increased during disease progression. Moreover, a significant correlation was found between WT1 expression levels, blast cell percentage, and the presence of cytogenetic abnormalities.

The patients received only a supportive therapy if necessary. After 6 months, 7 patients (2 RA, 3 RAEB I, 2 RAEB II) converted to AML. All of these patients showed at diagnosis an high WT1 expression level and a further elevation of WT1 expression after 6 months. Conclusion. WT1 expression has been previously reported to be increased also in myelodysplastic syndromes. In this study, the data obtained show that in most MDS, including a large percentage of RA and almost the total number of RAEB I and II, WT1 is expressed above the range observed in normal controls in BM and that its expression is directly correlated with the type of MDS. In addition, even within each subgroup, a strong association is present between the level of WT1 expression and the blast percentage and the presence of cytogenetic alterations. The identification of a molecular marker so able to establish the tendency of MDS to progression can be of great help in decision making for MDS patients. In conclusion we believe that WT1 can be introduced as a additional marker to the standard parameters already considered in risk assessment for MDS.

**0952**

**COST-EFFECTIVENESS OF CHELATION THERAPY WITH DEFERASIROX VERSUS DEFEROXAMINE IN TRANSFUSION-DEPENDENT MYELODYSPLASTIC SYNDROME**

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**Background.** Patients with myelodysplastic syndrome (MDS) frequently receive chronic transfusions, along with chelation therapy to prevent complications of iron overload. Deferoxamine is an effective iron chelator, but must be administered as an 8-12 hour infusion 5-7 times per week, leading to poor compliance and/or quality of life. Deferasirox is a once-daily oral chelator that has been shown to produce reductions in liver iron concentrations and serum ferritin similar to those obtained with deferoxamine. Aims. To evaluate from a US perspective the cost-effectiveness of deferasirox versus deferroxamine in patients with transfusion-dependent MDS. Methods. Data from a variety of published and unpublished sources were used to estimate the cost-effectiveness of deferasirox versus deferroxamine in MDS patients receiving frequent transfusions ($8 per year). As there are no long-term studies describing the complications of iron overload in MDS, we focused on the short-term (i.e., one year) cost and quality-of-life effects of chelation therapy. As comparative data for deferasirox versus deferroxamine was unavailable, we estimated the relative dose of deferasirox based on results for MDS patients in a non-comparative Phase II study (20 mg/kg/d). The relative dose of deferroxamine that would result in similar efficacy (21) was based on data from comparative studies in other transfusion-dependent anemias. We conservatively assumed that patients would be fully compliant with chelation therapy. Cost-effectiveness was measured in terms of the ratio of the difference (deferasirox versus deferroxamine) in costs of chelation therapy to the difference in quality-adjusted life years (QALYs) over one year. Unit costs of deferoxamine and deferasirox were based on US wholesale acquisition costs. The cost of deferroxamine administration was based on estimated clinician charges for US patients with transfusion-dependent anemias. Utilities for MDS patients receiving transfusions were based on published data for patients with anemia from metastatic cancer. The difference in quality of life for deferasirox versus deferoxamine was based on a study that used time-trade-off methods to estimate community-based preferences for oral versus intravenous chelation. Results. One year of treatment with deferasirox is estimated to result in a gain of 0.25 QALYs versus defereroxamine (0.78 versus 0.55). If the price of branded deferoxamine is employed, total annual costs are estimated to be $1,427 greater with deferasirox versus defereroxamine ($45,604 versus $44,177). The cost-effectiveness of deferasirox versus deferoxamine was $30,542. Cost-effectiveness of deferasirox versus defereroxamine was sensitive to the assumed dosages of deferasirox and defereroxamine and the costs and quality of life decrements associated with transfusion therapy. Conclusion. In patient with transfusion-dependent MDS, the cost per QALY gained with deferasirox versus defereroxamine is well within the range that is generally considered acceptable in the US.
DISPARITIES IN CRITERIA FOR INITIATING CHELATION THERAPY FOR IRON OVERLOAD IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES (MDS)

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Backgrounds. The Myelodysplastic Syndromes Foundation, Inc., a non-profit organization established by an international group of physicians and researchers to provide an ongoing exchange of information relating to MDS, conducted an international survey of practices and treatments of clinicians with expertise in diagnosing and treating MDS patients. Survey responses relating to iron overload were studied because it is estimated that more than 40% of MDS patients require regular red blood cell transfusions. Aims. To analyze survey data on current expert clinical management strategies for iron overload in MDS. Methods. Descriptive statistics were used to analyze The MDS Foundation’s 2004-2005 Practices & Treatment Survey responses received by email and fax through August 2005. The survey, developed by hematologists with expertise in MDS, was distributed to the MDS Foundation’s Centers of Excellence (48 US and 53 European and other academic medical centers). Results. Of the MDS Foundation’s 102 Centers of Excellence, 70 (38 US and 32 European and other non-US centers) responded to the survey. Responses indicate that a substantial proportion of MDS patients in all International Prognostic Scoring System (IPSS) risk groups are red blood cell transfusion-dependent: Low risk, 47%; Intermediate-1 risk, 58%; Intermediate-2 risk, 70%; High risk, 82%. Survey responses by European and other non-US centers revealed that an average of 87% of transfusion-dependent patients receive parenteral iron chelation therapy and that the criteria for initiating chelation therapy are not uniform. The number of transfusions was reported as a determining criterion by 47% of respondents, with a mean number of 36 transfusions. 15% of respondents reported that the number of transfusions was their sole criterion for initiating iron chelation therapy. Serum ferritin levels were reported as a determining criterion by 72% of respondents, with the following cutoff values:

<table>
<thead>
<tr>
<th>Ferritin concentrations for initiating chelation therapy.</th>
<th>% respondents using this cutoff as criterion</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;1000 ng/mL</td>
<td>35*</td>
</tr>
<tr>
<td>&gt;1500 ng/mL</td>
<td>17</td>
</tr>
<tr>
<td>&gt;2000 ng/mL</td>
<td>35</td>
</tr>
<tr>
<td>Other (&gt;3000, unspecified)</td>
<td>13</td>
</tr>
</tbody>
</table>

% respondents using this cutoff as criterion

32% of respondents indicated that serum ferritin was the sole criterion used. (97% indicated that they monitored ferritin levels in transfusion-dependent patients irrespective of chelation therapy.) Other criteria used to determine start of chelation therapy included age/life expectancy, MDS subtype, clinical signs of hemochromatosis, quantitative CT liver iron estimation, liver function, transferrin saturation >50%, BMT, anticipated chronic transfusion need, and logistical issues and insurance coverage. A combination of criteria was reported to be used by 38% of respondents. Conclusions. The decision for initiating chelation therapy in transfusion-dependent anemic MDS patients needs to be individualized because of the heterogeneous patient population. However, this data analysis suggests a need for standardizing select criteria, such as the number of transfusions and serum ferritin, for determining when to initiate iron chelation therapy.

ESSENTIAL THROMBOCYTHEMIA AND PREGNANCY: PRELIMINARY REPORT OF THE PREGNANCY COMMITTEE OF THE REGISTRO ITALIANO TROMBOCITEMIA (RIT)

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Backgrounds. Essential Thrombocytemia (ET) is diagnosed in the childbearing age in about 20% of patients. Fertility reduction and adverse outcome of pregnancy due to thrombotic or hemorrhagic complications are a matter of concern. Aims. To evaluate the outcome of pregnancy in a large series of patients in order to identify a possible guideline for the management of pregnancy in ET. Materials and Methods. The pregnancies observed in ET patients in seven Italian Hematological Centres from 1998 to 2005 were registered in the RIT. Results. Fifty-nine pregnancies occurring in 47 women (age 22-45 years) with ET diagnosed according to the WHO criteria were evaluated. None of these patients had a recognized thrombophilic abnormality other than ET. Besides 57 live births (66%), 7 first trimester losses (12.5%), 6 second trimester losses (10.7%), 2 still births (3.5%) and 5 voluntary abortions on social grounds were described; 3 pregnancies are ongoing. One case of IUGR and 6 premature births at weeks +26, +28, +32, +34, +34 and +36 respectively were reported. Maternal morbidity in this case series was absent. Thirty-six patients (61%) received Aspirin (100 mg) during the pregnancy and 9 out of them also received prophylactic LMWH for six weeks post-partum. Interferon α treatment was performed in 12 patients with a platelet count >1,000×10^9/L and considered at high thrombotic risk. The outcome of pregnancies in these 12 patients was the following: 12 live births (70.6%), 2 still births, 2 foetal losses (at weeks +8 and +28) and 1 ongoing. Overall there were 6 premature births at weeks +26, +32 + 33, +34, +34 and +36 respectively. Pregnancy outcome in the remaining group was the following: 22 live births (55%) 11 foetal losses (27.5%), 5 therapeutic abortions and 2 ongoing. Twenty-three pregnancies occurred among 18 women taking Interferon α (10 cases), Hydroxyurea (5 cases), Anagrelide (7 cases) and Busulphan (1 case). The pregnancy had the same outcome than in the overall population: 16 live births (69.5%), 5 foetal losses (21.7%), 1 premature birth (4.3%), 2 therapeutic abortions and 1 ongoing. Conclusions. These data confirm that foetal morbidity and mortality is not negligible in ET. Cytoreductive therapy with Interferon α seems potentially able to protect against foetal losses. Although normal pregnancies have been registered in patients who conceived during cytotoxic treatment, the adoption of effective forms of contraception throughout treatment is still strongly recommended. The epidemiological, clinical and biological data on pregnancy in ET obtained by the participating Centres are now object of prospective study by the RIT (CIMEMA project) which records the ET patients diagnosed in Italy since January 2004. Therapeutic options including antithrombotic treatment and cytoreductive therapy will be considered and a management plan for pregnancy in ET will be proposed.

EFFICACY AND SAFETY OF PEGYLATED INTERFERON α IN PATIENTS WITH POLYCYTHEMIA VERA: A PROSPECTIVE MULTICENTER PHASE II STUDY

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Backgrounds. Interferon α (IFN) is a therapeutic option for patients with polycythemia vera (PV) to control increased myeloproliferation. For pegylated formulations of IFN neither efficacy nor tolerability have been published in larger series of patients (pts) with PV. Aims. A phase II study has been conducted to investigate the antiproliferative effects and the safety of pegylated IFNα2b (PegIntron®) in PV patients. PegIntron® was administered subcutaneously with a starting dose of 50 µg/week. Dose escalation every six weeks to 100 and 150 µg/week or...
dose reduction was recommended according to response and toxicity. Pretreatment with one cytoreductive drug but not conventional IFN was permitted in addition to phlebotomy. Complete response (CR) was defined as a stable hematocrit ≤45% without phlebotomy, normalization of platelet counts and normal spleen size. Good partial response (PR1) was defined as reduction of phlebotomies and/or platelet counts and/or splenomegaly >50%, poor partial response (PR2) is the respective reduction between 25-50%. Results. Since February 2003, 49 pts (29 m / 20 f) with PV according to the WHO criteria have been enrolled. 21/23 pts investigated were positive for the JAK2(V617F) mutation. Follow up data are presently available from 37 pts with a median age of 59 (41-78) years and a median duration of therapy of 23 (2-36) months. At their most recent presentation, two pts received 5 µg Pegltntrax/wk, 14 pts 50 µg/wk, 6 pts 75µg/wk, 7 pts 100 µg/wk and 8 pts 150 µg/wk. A significant reduction of the initial platelet counts (<0.0001) was already seen after six weeks of therapy. By now, one patient achieved CR, 30 pts achieved PR1. Of these, 13 pts (35%) became free of phlebotomies for at least 6 months and achieved a normalization of platelet counts. However, normalization of spleen size has not been reached so far. Five pts achieved PR2, one patient had no change and another patient progressed to myelofibrosis after initial PR1. Side effects WHO grade I-II were observed in all 37 pts (arthralgia and flu-like symptoms in 84%, fatigue in 75%, injection site reactions in 57%, gastrointestinal toxicity in 35%, hepatotoxicity in 35%, depression in 37%, exanthema in 21%, alopecia in 16%, sexual dysfunction in 11% and hematotoxicity in 5%). WHO grade III depression or fatigue and arthralgia was observed in one patient each. Dose reduction due to side effects was necessary in 15 pts (40%). Pegltntrax was withdrawn in 12 pts (32%) after a median duration of therapy of 14 (2-28) months due to toxicity (3) or to continue therapy (9). Progression of the disease (n=1) or PV-related complications (n=2). In 12 out of 28 investigated pts a decrease of the PRV-1 expression during therapy was observed. Retrospective mutation screening for JAK2(V617F) will be performed in all patient samples available prior to therapy and during follow-up. Conclusion. Pegltntrax is an effective cytoreductive therapy for patients with PV. The reduction of the elevated platelet counts indicates the sensitivity to the drug in the early phase of therapy.

0957

RELATION BETWEEN PROPORTION OF GRANULOCYTE JAK2 (V617F) MUTANT ALLELES, CLINICAL PHENOTYPE AND DISEASE PROGRESSION IN CHRONIC MYELOPROLIFERATIVE DISORDERS

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Background. The occurrence of the somatic gain-of-function JAK2 (V617F) mutation in a hematopoietic stem cell can result in selective expansion and activation of its myeloid-lineage cell progeny, and consequently in a myeloproliferative disorder. By using sensitive assays, the JAK2(V617F) mutation was found in most patients with chronic myeloproliferative disorders (CMD) about half of those with essential thrombocythemia (ET) or chronic myeloid metaplasia (CMM) in hematopoietic cells characterizes a quite homogeneous category of hematologic disorders involving a transition from heterozygosity to homozygosity for the JAK2 (V617F) mutation in hematopoietic cells. From a clinical viewpoint, the present observations suggest that low proportions of mutant alleles (<25%) are mainly associated with thrombocytosis, intermediate proportions (25-75%) with erythrocytosis, and high proportions (>75%) with myeloid metaplasia and splenomegaly. Physiological and genetic modifiers are expected to further influence the clinical phenotype.

0958

HOMOZYGOSITY FOR JAK2V617F IDENTIFIES MPD PATIENTS WITH A MORE SYMPTOMATIC DISEASE. AN ITALIAN GIMEMA RETROSPECTIVE STUDY ON 989 PATIENTS

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Background. An acquired mutation in the JAK2 gene is found at different rates in patients with chronic myeloproliferative disorders (MPD); in about 20% of polycythemia vera (PV) or idiopathic myelofibrosis (IM), and in less than 3% of essential thrombocythemia (ET), the mutation is harboured in the homozygote status. This low frequency of homozygosity has prevented until now the elucidation of its impact on disease phenotype. Aims. The aim of this GIMEMA retrospective study was to evaluate homozygosity for JAK2V617F pointed to a subgroup of MPD patients with unique clinical characteristics. Design and Methods. In an Italian cooperative GIMEMA retrospective study, 989 MPD patients were enrolled from 11 hematology centers. The diagnosis of PV was made in 528 (53%), of ET in 400 (40%), while 224 (23%) were IM and 37 (4%) were post-PV/ET forms (PP/PTMM) of myelofibrosis. Diagnosis of PV or ET was made accordingly to either the PVSG or WHO criteria, while the Consensus Conference Criteria were used for IM. The only eligibility criteria for inclusion was the availability of a JAK2V617F mutational status determination according to the ASO-PCR and the BsaXI digestion method (Baxter, Lancet 2005). Results. In 177 patients (32%) were wild-type (WT), 520 (53%) were JAK2V617F heterozygote and 152 (15%) homozygote; among the latter, 81 were PV, 8 ET, 45 IM and 18 PP/PTMM, accounting for 25%, 2%, 20%, and 49%, respectively, of patients within each diagnostic group. Irrespective of their diagnosis, homozygote patients (152) had more frequent hematopoietic abnormalities than heterozygotes (520) (p<0.01). On the contrary, the presence of myelofibrosis or hepatosplenomegaly in the last 3 months was unchanged. The frequency of splenomegaly progressively increased from 40%, to 51% to 69% in WT, heterozygotes or homozygotes; similarly, the occurrence of puritus rose from 8% to 18% to 28%, and that of systemic symptoms from 25% to 30% to 58%. There were 351 thrombotic events, of which 251 were major events and 187 of the microvessels; major hemorhages were 45. There was a higher incidence of thrombosis in homozygotes (55%) than in heterozygotes (86%) or WT (26%), while there was no difference in hemorrhages. In PV and ET, homozygosity was associated with a greater risk of evolution into myelofibrosis (12% and 25%, respectively) compared to heterozygosity (2% and 25%) respectively; noteworthy, the highest frequency of homozygosity was recorded among PP/PTMM patients (49%). Finally, the frequency of patients overexpressing PRV-1 gene was greater among homozygotes (89%) than heterozygotes (69%) or WT (42%). Conclusions. This large survey of MPD patients, that allowed to evaluate 152 homozygote patients, supports the contention that the loss of wild-type JAK2 allele in hematopoietic cells characterizes a quite homogeneous category of patients with more symptomatic disease within each MPD diagnostic category. Assessment of JAK2V617F homozygosity may have a role in risk prediction and patient management.

GIMEMA MPD WP: Antonioli E, Guglielmelli P, Longo G, Bosi A (Firenze); Marchioli R (Pavia); De Stefano V, Leone G (Roma); Alimena G, Foà R (Roma); Ruggeri M, Rodighiero F (Vicenza); Specchia G, Liso V (Bari); Gerli G, Cattaneo M (Milano); Piazza R, Fogliani E (Monza); Carraoro MC, Rossi E (Milano); Tieghi A, Gugliotta L (Reggio Emilia); Marfisi MR (Chieti).
**0959 WHAT IS THE BEST STRATEGY FOR THE DIAGNOSIS OF POLYCYTHEMIA VERA?**

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Background. The diagnosis of polycythemia vera (PV) is based on major and minor, biological and clinical criteria (World Health Organisation or the Polycythemia Vera Study Group). These classifications do not use the recently described JAK2-V617F mutation as a criteria. Because of its high frequency in PV, and to a lesser degree, in other myeloproliferative disorders (MPD), the place of this new MPD marker among other biological tests has to be defined and validated. In most of the published reports on the frequency of the JAK2-V617F mutation in PV, patients were not at diagnosis. Methods. We report here the specificity and sensitivity of different approaches in the diagnosis of PV in a large cohort of 421 patients suspect of polycythemia, the JAK2-V617F mutation was studied in 124 patients. Results. Four hundred and twenty one patients (507 males, 114 females) were examined for persistent elevation of haematocrit (Hct). The RCM was measured in 365 patients and absolute erythrocytosis was confirmed for 290 patients. A high Hct (>56% in women and >60% in men) was always associated with high RCM (>125%) and was observed in 27% of patients. Depending on the criteria used, 208 patients (WHO criteria) or 167 patients (PVSG criteria) were diagnosed with PV. Thirty-two out of the 41 patients not diagnosed as PV following the PVSG criteria had endogenous erythroid colonies (EEC), a major WHO criterion. Altogether we observed 72 apparent erythrocytosis (AE), ie. patients with a RCM < 125%, 64 secondary erythrocytosis (SE) and 71 idiopathic erythrocytosis (IE). Following two diagnostic approaches recently proposed (A Toffori and J.J. Sprake, seminars in Hematology, 42:206-220, 2005), one based on RCM and oxygen saturation prior to the detection of JAK2-V617F, the other on serum EPO followed by the detection of JAK2-V617F and bone marrow biopsy, many patients were misdiagnosed 6% using the first approach, 2% using the second one. In term of expense, the first approach requests to test the RCM and the JAK2-V617F mutation in 73% and 45% of patients, respectively, whereas it is necessary to perform a bone marrow biopsy and the detection of the JAK2-V617F mutation in 54% and 96% with the second approach. The patients who presented a high Hct (>54% in males and >52% in females) and a thrombocytosis (>400 10^9/l) were all diagnosed as PV according to the WHO classification (n=81). Those with a high serum epo were all diagnosed as not PV (n=22). The RCM was reserved for the patients without the JAK2-V617F mutation. Conclusion: We propose a new approach, based on the JAK2 mutation as a first intention test associated with the blood cell count and the serum epo. The RCM should be reserved in JAK2-V617F negative patients.

**0960 PREVALENCE OF THE JAK2 V617F MUTATION IN PATIENTS WITH SPLANCHNIC OR CEREBRAL VEROUS THROMBOSIS AND WITHOUT SIGNS OF OVERT CHRONIC MYELOPROLIFERATIVE DISORDER**

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Backgrounds. Thrombosis of splanchnic or cerebral veins can develop in patients with chronic myeloproliferative disorders (CMD) such as polycythemia vera (PV) and essential thrombocythemia (ET); a CMD at very early stages not fulfilling diagnostic conventional criteria can account for a substantial proportion of all observed splanchnic venous thrombosis. Recently a somatic mutation (V617F) of the Janus kinase 2 (JAK2) was reported in a high proportion of patients with chronic myeloproliferative disorders (CMD). No data are available on the presence of the JAK2 mutation in patients with thrombosis of splanchnic or cerebral veins. Aims. To estimate the prevalence of the V617F JAK2 mutation in patients with idiopathic portal/splenic or cerebral vein thrombosis. Methods. We studied 111 adult patients (M/F 45/66, median age 40 years, range 18-79) with venous thrombosis of unusual sites: 12 with hepatic vein thrombosis (HVT), 60 with portal-mesenteric vein thrombosis (PMVT), 11 patients with splenic vein thrombosis (SVT) and 38 with cerebral vein thrombosis (CVT). No patient had clinical or laboratory conventional criteria for diagnosis of PV or ET. For comparative purpose 19 patients with overt CMD (6 with PV, 12 with ET, and 1 with idiopathic myelofibrosis IMF) were also investigated (M/F 9/11, median age 83 years, range 21-80); clinical manifestation was HVT in 5 patients, PMVT in 12, and CVT in 4. All patients were screened for the presence of the JAK2 mutation and thrombophilia (deficiency of antithrombin, protein C or S, factor V Leiden, prothrombin G20210A, hyperhomocysteinemia, lupus anticoagulant, anticardiolipin antibodies, anti-β2-glycoprotein antibodies). Results. The V617F JAK2 mutation was found in 17 (89.4%, 95% CI 68.6-97.0) of the 19 patients with overt CMD and thrombosis of unusual sites. In 9 cases (47.3%, 95% CI 27.3-68.2) the JAK2 mutation was the only putative risk factor, in the absence of thrombophilia or any circumstantial risk factor (oral contraceptives, surgery, puertermia, trauma). In the 111 patients without overt CMD a thrombophilic alteration was present in 36.9% (95% CI 28.5-46.2) of the cases. The JAK2 mutation was found in 13 (18.0%, 95% CI 10.8-28.4) of the 72 patients with HVT or PMVT and in 2 (5.1%, 95% CI 1.4-16.8) of the 39 patients with CVT. No difference was found in the prevalence of JAK2 mutation between patients with HVT and those with PMVT (p = 1.0). In 7 cases with HVT or PMVT (9.7%, 95% CI 4.7-18.7) without diagnosis of overt CMD the JAK2 mutation was the only putative risk factor. Conclusions. The V617F JAK2 mutation was detected in the large majority of the patients with overt CMD and splanchnic or cerebral venous thrombosis; in the absence of overt signs of CMD the mutation is still present also in a substantial proportion (18%) of patients with splanchic venous thrombosis and in a minority (5%) of patients with cerebral venous thrombosis. Screening for the V617F JAK2 mutation in such patients could identify CMDs at very early stages, having thrombosis as heralding symptom.

**0961 ASSAY OF THE STROMAL CELLS IN PATIENTS WITH CHRONIC MYELOPROLIFERATIVE DISEASES**

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Background. Chronic myeloproliferative diseases (CMD) is a group of malignant clonal disorders of haemopoietic stem cells. Malignant haemopoietic cells may exert influence on the stromal cells, which may result in alteration of the haemopoietic microenvironment. Aims. The purpose of the present study was to estimate functional activity of the haemopoietic microenvironment in CMD patients. We have studied the ability of bone marrow stromal cells from CMD patients to support proliferation and differentiation of cobblestone areas forming cells (CAFC) of normal individuals. Methods. Stromal and hematopoietic cells were obtained from bone marrow aspirates of 9 CMD patients (4 patients with idiopathic myelofibrosis, IMF and 5 patients with chronic myeloid leukemia, CML) and 6 normal individuals. We used the stromal feeder layers after irradiation (48 Gy): 3-4 week long-term cultures (LTC) and fibroblasts stimulated to osteogenic differentiation by dexamethasone (10⁻⁷M). The normal haemopoietic cells were seeded on the stromal cells of the CMD patients and of normal individuals. Functional activity of stromal cells of CMD patients was defined by the number of cobblestone areas-forming cells (CAFC) different stromal feeder layers in LTC and fibroblasts stimulated to osteogenic differentiation by dexamethasone (10⁻⁷(M)). The normal haemopoietic cells were seeded on the stromal cells of the CMD patients and of normal individuals. Functional activity of stromal cells of CMD patients was defined by the number of cobblestone areas-forming cells (CAFC) on different stromal feeder layers in LTC and fibroblasts stimulated to osteogenic differentiation by dexamethasone (10⁻⁷(M)). The normal haemopoietic cells were seeded on the stromal cells of the CMD patients and of normal individuals. Functional activity of stromal cells of CMD patients was defined by the number of cobblestone areas-forming cells (CAFC) on different stromal feeder layers in LTC and fibroblasts stimulated to osteogenic differentiation by dexamethasone (10⁻⁷(M)). The normal haemopoietic cells were seeded on the stromal cells of the CMD patients and of normal individuals. Functional activity of stromal cells of CMD patients was defined by the number of cobblestone areas-forming cells (CAFC) on different stromal feeder layers in LTC and fibroblasts stimulated to osteogenic differentiation by dexamethasone (10⁻⁷(M)). The normal haemopoietic cells were seeded on the stromal cells of the CMD patients and of normal individuals. Functional activity of stromal cells of CMD patients was defined by the number of cobblestone areas-forming cells (CAFC) on different stromal feeder layers in LTC and fibroblasts stimulated to osteogenic differentiation by dexamethasone (10⁻⁷(M)).

**11th Congress of the European Hematology Association**
GENE EXPRESSION PROFILING IN ESSENTIAL THROMBOCYTHESIA USING CDNA MICROARRAY TECHNOLOGY. PRELIMINARY RESULTS

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Background. Essential thrombocythemia (ET) is a chronic myeloproliferative disorder (CMPD) lacking specific molecular markers. Consequently, its diagnosis is based on exclusion of other CMPD and secondary thrombocythemia. Aim. The aim of the study was to characterize the gene expression profiling in ET using cDNA microarray technology, specially analyzing the implication of the JAK-STAT signaling pathway. Patients and Methods. Peripheral blood granulocytes obtained from 20 ET patients diagnosed according to the PVSG criteria (1997), who had not received platelet-lowering therapy, were isolated. Good quality RNA (RIN≥7) was hybridized competitively to the RNA granulocytes obtained from 1 ET patient and 1 normal volunteer. Duplicate morphology was performed with dye-swap to control for possible differences in the incorporation rate of the two fluorochromes. Oligonucleotide cDNA microarray expression profilings were obtained using 44K whole human genome oligo microarrays (Agilent Technologies, Palo Alto, CA), comprising 41,000 oligonucleotides. Fluorescent images were obtained using an Agilent G2565BA scanner and Genepix 6.0 (Axon Inc.) was used to extract data from the image and analysis was performed using the R-package. Results. From 41,000 oligonucleotides covered in the Agilent platform, an homogenous TE signature was obtained in 17 out of 20 patients studied. This signature was composed of 124 genes that were found to not be more than 2-fold up-regulated and 14 genes 2-fold down-regulated, in relation to control granulocytes. The other 3 patients showed a distinct expression profile and different to each other. Of the 124 up-regulated genes, 101 had an assigned function, being the immune response the most implicated, with 29 genes overexpressed. In addition, cellular movement (28 genes) and hematological system development and function (28 genes) were also involved. The most important involved network comprised 35 genes, mainly CXCL2, CCL3, PTGS2, CCL3L1, GCH1 and TNFAIP3, which mediate immune and inflammatory responses. Two other networks also implicated included cellular growth and proliferation, cellular movement and hematopoietic system development and function (15 genes, being the most important DUSP6, MAP3K5, WAS and TNFR2) and cellular movement and cell cycle, and cell cycle, respectively (13 genes, among them DNMT1). An interesting group of up-regulated genes was the chemokine family, involved in cell movement, chemotaxis, signal transduction and cell communication (CXCL2, CCL4, CCL5, CCL20, CCL23). Regarding to the 14 down-regulated genes, 9 of them had an assigned function and included transcription factors (SOX4, ZNF217), genes involved in immune response (C4BPA, C1QG), cellular protein metabolism (TBCG, SOLH), cellular localization and intracellular transport (APF3M1), PRV-1, c-MPL and TPO gene expression was not affected in any of the 20 patients studied. Interestingly, only one gene (CXCL2) involved in the JAK-STAT signaling pathway was affected. No differences regarding expression patterns were found between ET patients with and without the JAK2 V617F mutation. Comments. Our preliminary results have shown an homogeneous expression pattern in 17/20 ET patients. It is remarkable that an important number of genes were up-regulated, most of them being implicated in the immune response, cellular movement and hematological system development and function.

MOLECULAR ANALYSES IN FAMILIAL AND SPORADIC CONGENITAL PRIMARY ERYTHROCYTOSIS

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Backgrounds. The only molecularly characterised type of primary familial and congenital erythrocytosis/polycythemia (FPCP) is caused by dominant mutations in the erythropoietin-receptor gene (EPOR). EPOR mutations are estimated to account for 12-15% of cases with congenital primary erythrocytosis. So far, at least fourteen different EPOR mutations have been described, eleven of them leading to a truncation of the intracellular part of the receptor, resulting in hypersensitivity of erythrocyte progenitors to circulating Epo. The majority of previously reported mutations has not been identified in additional patients outside of the index family. Aim. Search for the underlying cause of familial and sporadic congenital primary erythrocytosis of so far unexplored origin: 1. Analysis of EPOR to identify unknown mutations or any of the previously reported mutations if occurring independent from the original family. 2. In patients without EPOR changes, exclusion of a somatic or germline JAK2 V617F mutation, which has been previ-ously detected in patients with polycythemia vera (PV) and other myelo-proliferative disorders. Method: 16 patients (age range 5-66 years) with a serum Epo level of < 10 mU/mL have been included in this study, 3 of them being related (a mother and two of her sons). P. vera was excluded according to FVSG or WHO diagnostic criteria. Sequencing analysis of coding regions and intron/exon boundaries of the EPOR gene was performed on genomic DNA. An allele-specific PCR was used to either confirm or reverse the JAK2 V617F mutation. Results. An EPOR mutation 1453G→A creating a termination signal at codon 459 (1P4G39ter) was
found in a 5 year old Spanish girl. Her parents and her brother do not present this mutation and have normal blood counts. Another EPOR mutation (EPOR 1414C→G, Tyr426ter) was detected in the multimember family case. Interestingly, the mother currently presents with normal hemoglobin and hematocrit levels (only mild microcytosis). She had been included in the study because of her affected sons but also because of her history of erythrocytosis during childhood and adolescence. This change of the clinical presentation is of particular interest since the original identification of this mutation in first PCFP family had been complicated by the fact that one family member with the mutation was apparently clinically unaffected. None of the patients presented the JAK2 V617F mutation. Conclusion: EPOR gene mutations causing either sporadic or familial primary congenital erythrocytosis are found in patients of various ethnic origins. Considering the fact that 3 of the 16 patients were from one family, the previously reported prevalence of EPOR gene mutations in the group of primary erythrocytosis of about 15% is confirmed by this study. The mutation EPOR 1414C→G previously described was independently detected in a second family and is associated with a variable phenotype. The exploration of the underlying pathophysiological mechanisms may contribute essentially to the knowledge about erythropoiesis’ regulation. The PV-characteristic mutation JAK2 V617F does not seem to play a role in congenital primary erythrocytoses.

0965
THE LEVELS OF JAK2V617F RNA DICTATE THE CLINICAL PHENOTYPE IN POLYCYTHEMIA VERI AND IDENTIFIES PATIENTS WITH MORE SYMPTOMATIC DISEASE
Background. The occurrence of an unique JAK2 V617F mutation in phentotypically distinct chronic myeloproliferative disorders (MPD), including polycythemia vera (PV), essential thrombocythemia (ET) and idiopathic myelofibrosis (IM), suggests that other genetic events/gene modifiers might be involved. Aims. As an approach to unravel significant associations between phenotype and the JAK2 mutation, we have correlated the levels of JAK2V617F RNA with clinical and laboratory characterstics at the diagnosis in 63 patients with polycythemia vera (PV) and 115 with essential thrombocythemia (ET), as diagnosed according to the WHO criteria. Methods. Wild-type and mutated JAK2 RNA levels were determined by an amplification-refractory mutation sequencing (ARMS) PCR assay on granulocytes, and expressed as the percentage of mutated JAK2 RNA over total (Vannucchi AM et al. Leukemia, In press). Results. 53/65 PV patients (84%) and 76/115 (66%) presented detectable levels of JAK2V617F RNA; the amount of mutated RNA was higher in PV than in ET granulocytes (median 52% and 12.5%, respectively; p<0.0001). In PV patients, the hematocrit and white blood cell count were significantly related to the amount of mutated RNA, while there was an inverse relationship with MCV and platelet count. None of these correlations were found in mutated ET patients, in whom the correlation with mutated RNA levels in ET. Even when the analyses were restricted to those PV patients who showed RNA levels in a range similar to that observed in ET (1-55%) the above correlations were maintained, thus ruling out that these effects might be simply ascribable to the overall higher load of JAK2V617F RNA in PV than in ET patients. Among PV patients with JAK2V617F mutation, the frequency of splenomegaly, of therapy (flebotomies and antiplatelet agents) was significantly increased over wild-type patients, but again not in ET pts. On the other hand, in both PV and ET JAK2 V617F mutated patients there was a greater frequency of EEC and overexpressed PRV1 gene, while there was no difference in CD34+ cell count in the peripheral blood. The percentage of high-risk patients was higher among mutated than wild-type ones (63% vs. 27%, p=0.003) if patients were all considered together, but did not reach the significance level in the ET group alone (62% vs. 36%, p=0.07); on the contrary, in PV there was a progressive increase in the percentage of high-risk patients according to the amount of mutated RNA (10% in wild-type, 24% in patients with 1-25% JAK2V617F RNA, and 66% among those showing 26-100% JAK2V617F RNA). Conclusions. By quantifying the amount of JAK2V617F RNA in granulocytes, we documented a gene dosage-effect in PV, but not in ET, suggesting that the JAK2 mutation dictates the clinical phenotype in PV patients while additional genetic or host factors modulate the disease presentation in ET. Also of note, the levels of JAK2 V617F RNA identified PV patients with more symptomatic disease in terms of blood abnormalities, therapy requirement and high-risk category.

0966
DIAGNOSIS OF ESSENTIAL THROMBOCYTHYEMIA: THE USEFULNESS OF JAK2 V617F MUTATION DETECTION IN PLATELETS RNA
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Backgrounds. The discovery of the JAK2 V617F mutation has profoundly modified the diagnosis of myeloproliferative diseases (MPD). In essential thrombocythemia (ET), the most frequent MPD, the mutation has been found in 30 to 57% of cases studying neutrophil’s DNA. Aim: In this study we aimed to assess the most informative cellular fraction for the JAK2 V617F detection. Patients and Methods. We explored a cohort of 260 consecutive patients referred to our institution with a suspected diagnosis of ET. We studied neutrophils and bone marrow mononuclear cells at the DNA level and platelet’s RNA. The detection of the mutation consisted in a real time PCR on LightCycler followed by a melting curve analysis (sensitivity 2-4%), allowing a semi-quantitative estimation of mutated/wild type allele. Bone marrow culture assays for endogenous erythroid and megakaryocytic colony formation (ECC, EMC) were performed in 146/260 patients. Results. The mutation was found in 141/260 (54%) patients. In 82 patients both neutrophils and platelets were studied. In 52/82 patients the mutation was detected in neutrophils and 40/82 in platelets (p=0.18). Thus 8 patients were detected only in platelet’s RNA. Using an optimised assay (sensitivity 0.8%) all of them were found mutated in neutrophils. However these patients were more easily detectable in platelets. Furthermore, 16/52 (50%) had no more than 10% JAK2 V617F allele in neutrophils. In conclusion only 2/57 PV patients had the same profile. In 27 patients studied both in platelets and bone marrow, no difference was found for JAK2 mutation status. Bone marrow cultures revealed ECC or EMC in 64/74 (86%) of JAK2 mutated samples. ECC or EMC were also found in 15/46 (33%) JAK2 V617F negative samples in platelets or bone marrow. Conclusion. In ET, JAK2 V617F mutation is more easily detected in platelets. The presence of ECC or EMC in JAK2 non mutated cases supports the idea of another underlying molecular defect. Therefore bone marrow cultures and morphology remain useful for the diagnosis of these MPDs.

0967
UPDATE OF THE GERMAN ESSENTIAL THROMBOCYTHEMIA (ET) STUDY: INCIDENCE OF COMPLICATIONS DURING LONG-TERM FOLLOW-UP
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The German ET-Study is a prospective, randomized multicenter trial with 51 participating centers recruiting 123 patients until 1999. Patients were stratified according to a previous history of ET related complications or a platelet count > 1500 G/l in high or low-risk ET patients. ET patients were regarded as high-risk ET patients, if there has been a previous history of ET related complications or if the platelet count was > 1500 G/l. These patients were randomized either to interferon α (IFN) or hydroxyurea (HU). Low risk ET-patients were defined as ET patients with no ET related symptoms and a platelet count < 1500 G/l. These patients were observed until ET related complications did occur or until the platelet count increased above 1500 G/l. In total 123 patients with a newly diagnosed ET according to the PVSG criteria and no prior cytoreductive treatment were recruited. Out of these 123 patients, 55 had a high-risk ET and were randomized to either HU (n=27) or IFN (n=28). The remaining 68 patients had a low-risk ET. After a median follow-up of 6 years (range 1-10 years) 13 low risk ET patients developed ET-related symptoms (8 thromboembolic episodes and 5 microcirculatory disturbances). This resulted in a total complication rate of 3.5% per 100 patient-years and in a rate of 2.0% per 100 patient-years for thromboembolic complications alone. No major bleedings were observed. ET-related complications were significantly dependent on age (age ≥ 60 years, p=0.001) or the presence of ≥ 2 cardiovascular risk factors (p=0.006). After a median follow-up of 6.6 years (range 0.2-11.1 years) the total complication rate in high risk patients was 3.2% per 100 patient-years for IFN treated patients and 6.7% per 100 patient-years for HU treated patients (p=0.5). Age ≥ 60 years (p=0.01) or the presence of ≥ 2 cardiovascular risk factors (p=0.026) were associated with a significant higher risk of ET related complications in all high risk patients. After 3 years, half of the
IMATINIB-MESYLATE THERAPY FOR SYSTEMIC MASTOCYTOSIS: RELATIONSHIP TO C-KIT MUTATIONAL STATUS

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Aims. Since c-kit is a transmembrane receptor-type tyrosin kinase, we aimed to test the hypothesis of whether an inhibitor block-

ing constitutive c-kit activation, such as imatinib, could have therapeu-
tic activity in SM and whether c-kit mutational status could have impor-
tance for response. Methods. We report on nine patients treated with imatinib who met the major classification criteria for SM, who were symptomatic and who had a biopsy-proven evidence of disease. Six of them were male and three female, aged range from 35 to 76. Organ involvement included skin, bone, stomach, bowel, bone marrow, spleen, liver or lung and was frequently combined. Two patients had elevated eosinophils in their peripheral blood (45% and 19%, respectively). All patients resulted to be negative for the FIP1L1-PDGFRA fusion transcript, which characterizes hypere-
osinophilic syndromes (HES) with high sensitiveness to imatinib. The drug was given at the dose of 400 mg/die for a median period of 3 months (range 1.5–5 months). Results. Before therapy, mastocyte cells from six patients were found positive for D816V mutant of c-kit. None of these patients achieved significant clinical benefits from imatinib ther-

"0968 A PHASE II STUDY OF NILOTINIB (AMN107), A NOVEL TYROSINE KINASE INHIBITOR, ADMINISTERED TO PATIENTS WITH SYSTEMIC MASTOCYTOSIS"

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Background. Systemic mastocytosis is a clonal disorder characterized by constitutive activation of c-Kit based on point mutations and is char-
acterized by mast cell infiltration of extracutaneous organs. Nilotinib is a novel aminopyrimidine which potently inhibits Bcr-Abl, as well as the PDGF-R and c-Kit kinases. Aim. This study was designed to evaluate the safety and efficacy of nilotinib administered at an oral dose of 400 mg twice daily. Methods. This is a Phase II, open-label study of SM patients with specific disease criteria and with a clinical indication for treatment. Results. Preliminary data are available for the first 23 (11 f, 12 m) out of 55 patients currently enrolled in the study. The median age is 49 (range 33–78) years and the median time from diagnosis of SM was 27 (range 1 to 292) months. Of the patients with data available 17 pts had a c-kit D816V mutation in bone marrow cells or extracutaneous organs. The median exposure to nilotinib was 144 days. Treatment is ongoing for 18 (75%) patients, 5 (22%) have discontinued, and 5 (15%) for adverse events and withdrawal consent. There were three (15%) responses report-
ed (2 incomplete remission and 1 minor response) based on serum tryptase, bone marrow mast cell counts and improvement of clinical symptoms. Baseline mutation data are available for 2 of the 3 respond-
ing patients and revealed the c-kit D816V mutation. Anemia was report-
ed in 2 (9%) patients. Adverse events occurring in ≥10% of patients included headache 52% (n=12), fatigue 39% (n=9), nausea 35% (n=8), vomiting, pruritis 30% (n=7 each), muscle spasms 26% (n=6), diarrhea, upper abdominal pain, rash 22% (n=5 each), dizziness, extremity pain, dyspnea, myalgia, increased ALT 17% (n=5 each), bone pain, abdom-
inal pain, cough, hand eczema, purulent rash 3% (n=1 each) and hypotension 5% (n=1 each). Overall Grade 3/4 adverse events included headache, pruritis, hypotension, dyspnea, myalgia, increased ALT 9% (n=2 each), fatigue, muscle spasms, diarrhea, dizziness and extremity pain 4% (n=1 each). There were no deaths. Summary/Conclusions. These data suggest that nilotinib has clinical activity and an acceptable safety and tolerability profile in patients with systemic mastocytosis.
detected in 14, only 1 of whom had a secondary MMM (p=0.02); such patients showed higher values of leukocyte count (p=0.018), CD34 count (p=0.03), serum LDH (p=0.02) and spleen size, a higher frequency of severe anemia (hemoglobin<9g/dl; p=0.02), higher SDF1x plasma levels (p=0.04), lower percentage of CD34+ CXCR4+ cells, higher percentage of CD34+ intraCXCR4 cells and a trend to a lower bone marrow cellularity as compared with both BM and SDF1x-3'A AG or AA patients. Sims. To phenotype differences between the GG and the AG/AA genotypes were detected both in idiopathic and secondary MMM patients, and also in the patients with mutated JAK2. Conclusions. The SDF-1-3'A polymorphism is highly frequent in secondary MMM and influences MMM phenotype, favoring a less intense myeloproliferation and less severe anemia.

0971.
TOPOGRAPHY OF INTRAMEDULLARY HEMATOPOIESIS IN MYELOFIBROSIS WITH MYELOID METAPLASIA: RELEVANCE OF 99TC-BW250/183 IMMUNOSCINTIGRAPHY

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Backgrounds. Myelofibrosis with myeloid metaplasia (MMM) is a rare chronic myeloproliferative disease characterized by both myeloproliferative and myelodepressive features: myeloproliferation typically includes enhanced spontaneous mobilization of hematopoietic progenitor cells (HPC) from the bone marrow (BM) and their homing into extramedullary sites (mainly spleen); myelodepletion results from exhaustion of both BM and SDF1x-3'A AG or AA patients. Sims. To investigate the extent and distribution of hemopoiesis in MMM patients and to capture its relationship with BM fibrosis, HPC mobilization and clinical severity. Methods. Immunoscinfigraphy employing a dual-head camera was performed 120-260 minutes (median 180 minutes) after administration of 553-830 MBq (median 700 MBq) 99mTc-BW250/183, corresponding to 0.3-0.5 mg. Hemopoietic function in the central compartment (sacrum) was described by subtypes (normal, increased or decreased) and by the sacrum-to-so soft tissue uptake ratio (UR) (Huic et al., J Nucl Med 1997). The degree of peripheral BM displacement (limbs) was described through 5 types (I to V) (Huic et al., J Nucl Med 1997). Chi-square and ANOVA tests were adopted for descriptive statistics. Results. Twenty-three MMM patients (10 females, median age 55 years) were studied. Eleven patients showed a reduced uptake by the central BM compartment: they had a higher WHO fibrosis grade (p=0.008), lower hemoglobin values (p=0.012) and lower platelet counts (p=0.007) than patients with a preserved central compartment. Patients with an exhausted central compartment at immunoscintigraphy also showed a significantly higher mobilization of HPC into peripheral blood: CD34+ count was 0.86% in patients with a depressed central compartment versus 0.12% in those with a preserved one (p=0.029). Accordingly, immature myeloid cells or blasts (9.5% versus 0.5%; p=0.005), spleen size (9.3 vs 2.4 centimeters from costal arc; p=0.007) and LDH values (1168 vs 566 IU/l; p=0.011) were significantly higher. Among the patients with a depressed central compartment, those who lost also peripheral BM function (type V) showed a more severe myelodepletion and more intense HPC mobilization. On the opposite side, among the 12 patients with a preserved central compartment, the 3 ones with a mild peripheral marrow displacement (type I) showed absent or mild fibrosis (WHO 0-1), elevated platelet counts, normal to high hemoglobin values, minimally enlarged spleens and no hints of increased HPC mobilization (CD34+ <0.1%; no blasts; ≤1% immature myeloid cells). From these data it appears that progressive fibrosis and exhaustion of central BM is accompanied by a gradual displacement of hemopoiesis into the peripheral bone compartment and spleen and by derangement of HPC trafficking. Conclusions. BM immunoscintigraphy tracks MMM clinical features and may help staging patients, understanding the biology of the disease and targeting therapies.
**0973**

**FAMILIAL CHRONIC MYELOPROLIFERATIVE DISORDERS: CLINICAL PHENOTYPE AND JAK2 (V617F) MUTATION STATUS**


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**Background.** Philadelphia (Ph)-negative chronic myeloproliferative disorders (CMD) include polycythemia vera (PV), essential thrombocythemia (ET) and chronic idiopathic myelofibrosis (CIMF). CMD are acquired diseases due to a somatic stem cell mutation leading to clonal expansion of myeloid precursors. A gain-of-function mutation of the Janus kinase 2 (JAK2) gene has been recently recognized as a pathogenetic event of CMD. Besides sporadic cases, one or several CMD may affect different relatives of the same family, namely familial CMD. The probability that two CMD occur in the same family as independent events is really low (estimated annual incidence: $10^{-4}$). This suggests the presence of genetic predisposition for somatic mutations leading to CMD-like syndromes in families. Although familial cases carrying JAK2 (V617F) mutation have been reported, the frequency of this mutation in CMD families and its role in disease-causing remain to be defined. Aims. The aim of this study was to evaluate the clinical features and outcome of familial chronic myeloproliferative disorders, to assess the frequency of JAK2 (V617F) within familial cases, and to define its role in disease-causing. Patients and Methods. Sixteen pedigrees were evaluated for clinical and molecular studies. Pedigrees included 11 families with an homogeneous phenotype (polycythemia vera in 8; essential thrombocytopenia in 3), and 5 with a mixed CMD phenotype. Collecting DNA from granulocytes and T lymphocytes, we detected JAK2 (V617F) by use of quantitative mutation-specific polymerase chain reaction and X-chromosomal clonality markers HUMARA, PGK, and IDS. Results. Clinical features at diagnosis and outcome did not differ between familial and sporadic CMD. JAK2 (V617F) ranged from 5.3 to 91.5%: the higher value being detected in post-polycythemia myelofibrosis. Distribution of mutant JAK2 within the same pedigree displayed an homogeneous pattern (5 families), or a discordant one (4 families). T cells DNA did not carry mutant alleles. All clonal CMD females, except one with ET, were JAK2 (V617F)-positive. One polyclonal ET was JAK2 (V617F)-positive (low gene dosage: 5.3%). One PV patient was polyclonal and JAK2 (V617F)-negative. Screening of healthy relatives identified 2 subjects with early-polycythemia. Conclusions. These data show that patients with familial CMD have clinical features and outcome overlapping with those of sporadic cases. An extended study of the pedigrees of CMD patients is warranted to ascertain the real frequency of familial cases. JAK 2 (V617F) is a somatic mutation that at least in a portion of familial patients with ET or CIMF does not appear the disease-initiating event.

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**0974**

**SPANISH REGISTRY OF ESSENTIAL THROMBOCYTHEMIA**

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**Background.** The RETE (Registro Español de Trombocitemia Esencial) is an open, retrospective and multicenter registry of patients with essential thrombocytemia (ET) treated with Anagrelide, designed and promoted by GEMFIN (Grupo de Estudio de Enfermedades Mieloproliferativas Filadelfia Negativas). Aims. The aim of the registry was to assess retrospectively the efficacy and safety of Anagrelide in newly diagnosed ET patients, as well as in those intolerant/refractory to their current therapy. Patients and Methods. 411 ET patients from 54 Centers were included. ET was diagnosed according to PVSG criteria (1997). Risk groups were defined as follows: high risk (patients with an age above 60 years and/or history of thrombosis), low risk (patients with an age below 60 years, no history of thrombosis and platelet count < 1.5x10^9/L) and intermediate risk (patients that belonged neither to high nor low risk groups). Response to treatment was defined as complete remission (CR) when a plateau count equal or less than 400 x 10^9/L was achieved; partial remission (PR) when the platelet count was between 400 and 600x10^9/L and no response (NR) when platelet count was > 600 x 10^9/L. All these data and adverse events were collected along 2004 and sent for blind analysis by two external data manager. Results. The median age at diagnosis was 50 years (14-92) and 25% of patients were females (61.6%). Patients were stratified at diagnosis in the following risk groups: 167 (40.6%) high-risk, 97 (23.6%) intermediate-risk and 147 (35.8%) low-risk. At presentation, 114/411 (27.7%) manifested thromboembolic complications and 73/411 (17.7%) showed bleeding events. Evolution to myelofibrosis, polycythemia vera and acute leukemia/MDS was observed in 20, 4 and 9 patients, respectively. The reason/s to initiate Anagrelide were thrombocytosis (n=282; 68.6%), tachycardia (26.7%), palpitations (9.7%), oedema (17%), diarrhea (14%), vertigo (13%) and dyspepsia (11%). Discontinuation of Anagrelide was reported in 36.2% of patients and was due to headache (61%), tachycardia (26%), palpitations (52%), oedema (17%), anemia (17%), diarrhea (14%), vertigo (13%) and dyspepsia (11%). Discontinuation of Anagrelide was reported in 36.2% of patients and was due to headache (61%), tachycardia (26%), palpitations (52%), oedema (8%), and anemia (8%). The incidence of thrombosis and hemorrhage observed during Anagrelide treatment was 5.6% and 6.8%, respectively. Summary. RETE study showed: a) a high hematological response rate in Anagrelide naive and previously treated patients, (56.4% and 78.7%, respectively) and, b) an incidence and type of adverse events in agreement with those reported.

Funding. Shire Ibérica.
Acute myeloid leukemia and myelodysplastic syndromes - Clinical

0975 CYTOGENETICS AND AGE ARE THE MAIN DETERMINANTS OF OUTCOME IN INTENSIVELY TREATED ACUTE MYELOID LEUKEMIA PATIENTS OLDER THAN 60 YEARS: RESULTS FROM AMLSG TRIAL AML HD98-B

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Backgrounds. Karyotype at diagnosis provides the most important prognostic information in younger adults with acute myeloid leukemia (AML). However, there are few data available looking in particular at patients (pts.) above 60 years of age. Aims. Evaluation of the prognostic value of cytogenetics and additional variables in elderly AML patients.

Methods. We prospectively analyzed 581 elderly pts. with newly diagnosed AML. Chromosome banding was performed using standard techniques. To improve cytogenetic diagnostics, all specimens were analyzed by FISH using a comprehensive DNA probe set for the detection of the most relevant AML-associated genomic aberrations: inv(3)(q21q26), t(8;21), t(9;22), t(11q23), t(15;17), inv(16)(p13q22), del(17q), +8q, +11q, abn(12p), del(13q)/+13q, del(17q), del(20q), +21q, +22q, del(Xq). All pts. were treated within the AMLHD98B treatment trial and received intensive induction and consolidation therapy. Pts. exhibiting a t(15;17) received an age-adjusted AIDA-regimen. Median follow-up time was 57 months. The median age was 67 years (range 60-85 years). Results. 161 pts. had a normal karyotype (45%); 48 pts. (13%) exhibited the balanced translocations t(8;21) (n=12), inv(16) (n=14), t(15;17) (n=11), or t(11q23) (n=11); in absence of these balanced aberrations, and 61 pts. a complex karyotype (a3 aberrations; including 44 pts. with 5 or more rearrangements). Analyses were limited to pts. with normal karyotype. Pts. exhibiting a t(15;17) showed a significantly better CR (29%) and OS (55%), whereas pts. with the other balanced translocations (t(8;21), inv(16), t(15;17), inv(16)(16,16), +8q, +11q, abn(12p), del(13q)/+13q, del(17q), del(20q), +21q, +22q, del(Xq)). All pts. were treated within the AMLHD98B treatment trial and received intensive induction and consolidation therapy. Pts. exhibiting a t(15;17) received an age-adjusted AIDA-regimen. Median follow-up time was 57 months. The median age was 67 years (range 60-85 years). Results. 161 pts. had a normal karyotype (45%); 48 pts. (13%) exhibited the balanced translocations t(8;21) (n=12), inv(16) (n=14), t(15;17) (n=11), or t(11q23) (n=11); in absence of these balanced translocations, 73 pts. exhibited a single aberration, 179 pts. two aberrations, and 61 pts. a complex karyotype (a3 aberrations; including 44 pts. with 5 or more rearrangements). Analyses were limited to pts. with normal karyotype. The limited backward selected Cox-model for OS (t(15;17) excluded) revealed two risk groups: standard-risk (normal karyotype, t(8;21), inv(16), t(11q22), +8 and +11 in absence of a complex karyotype) and high-risk (all other aberrations). The second main determinant for prognosis was age with a cut point at 70 years defined by maximally selected log-rank statistics (p<0.001). Stratification of the patients according to cytogenetic risk group and age as dichotomized variable resulted in 5 prognostic groups: i) APL CR 75%, OS 55%, ii) <70 yrs./standard risk CR 62%, OS 24%, iii) <70 yrs./high risk CR 21%, OS 6%, iv) >70 yrs./standard risk CR 89%, OS 5%, v) >70 yrs./high risk CR 15%, OS 2%. Conclusions. Our risk classification system based on cytogenetics and age identified a large proportion of elderly patients with AML who did not benefit from intensive chemotherapy.

0976 5-AZACITIDINE INDUCES REMISSIONS IN PATIENTS WITH TRANSFUSION DEPENDENT MYELOPROLIFERATIVE DISEASES AND IN PATIENTS WITH ACUTE MYELOID LEUKEMIA refractory to or not eligible for intensive chemotherapy

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Backgrounds. Epigenetic modulation of gene function is a powerful cellular mechanism. An association between methylation of the p15 ink4b gene promoter and risk for acute myeloid leukemia (AML) transformation in myelodysplastic syndrome (MDS) has been suggested. The DNA hypomethylating agent 5-aza-2′-deoxycytidine (azacitidine) induces remarkable responses in 60% of patients with AML who are refractory to or not eligible for intensive chemotherapy as well as in patients with myeloproliferative diseases (MPD). The current study was conducted to demonstrate the clinical efficacy and safety of azacitidine in elderly patients evaluable for safety. We combined DA induction (daunorubicin 60 mg/m² d 1-3 and cytarabine 100 mg/m²/d by IV CI) on day 1-7 and post-remission HD-ARAC (cytarabine 3 g/m²/3h q 12h, d1,d5,5 for 3 cycles) plus PKC412 in newly diagnosed FLT3 mutated (FLT3mut) and FLT3 wild type (FLT3WT) AML patients < 60 years old in a Phase Ib trial to investigate toxicity and efficacy. Results: Of earlier experience using PKC412 100 mg po bid were reported previously (Colles et al., ASH 2005). This is an updated report of 22 patients treated with PKC412 at a reduced dose of 50 mg po bid given on day 8-21 (arm 1) or day 1-7, 15-21 (arm 2), plus DA and HD-ARAC. Eight out of 23 patients (35%) were FLT3mut, and 15/23 (65%) were FLT3WT as determined by D-HPLC. Results. None of the 19 patients evaluated for toxicity had drug-related death; the most common non-hematologic toxicities were transient elevations of glucose (16%), AST (16%), bilirubin (11%), ALT (11%), and decreases in potassium (21%), phosphate (11%) and calcium (11%); no grade 3 or 4 nausea, vomiting or pleural effusion were recorded. Twenty-three patients were evaluable for response: 9/12 (75%) achieved CR in Arm 1 and 9/11 (82%) achieved CR in Arm 2. Seven out of 11 (63%) FLT3mut patients and 11/15 (73%) FLT3WT patients achieved CR. FLT3mut patients achieved CR at a significantly higher rate (p=0.047) than FLT3WT patients.

0977 PHASE II STUDY OF PKC412, AN ORAL FLT3 KINASE INHIBITOR, IN SEQUENTIAL AND SIMULTANEOUS COMBINATIONS WITH DAUNORUBICIN AND CYTARABINE INDUCTION AND HIGH-DOSE CYTARABINE CONSOLIDATION IN NEWLY DIAGNOSED PATIENTS WITH AML


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Backgrounds. Activating mutations in FLT3 (fms-like tyrosine kinase), either an internal tandem duplication (ITD) in the juxtamembrane region or a point mutation in the activation loop, occur in leukemic blasts from 25-55% of AML patients, are associated with poor prognosis, and represent an attractive therapeutic target. PKC412 is a multi-targeted kinase inhibitor which has clinical activity in mutant (reduction in peripheral blasts in 70%) and wild type (reduction in peripheral blast in 50%) AML, but rarely produces remissions (Stone et al., Blood 2005). Aims and Methods. We combined DA induction (daunorubicin 60 mg/m² d 1-3 and cytarabine 100 mg/m²/d by IV CI) on day 1-7 and post-remission HD-ARAC (cytarabine 3 g/m²/3h q 12h, d1,d5,5 for 3 cycles) plus PKC412 in newly diagnosed FLT3 mutated (FLT3mut) and FLT3 wild type (FLT3WT) AML patients < 60 years old in a Phase Ib trial to investigate toxicity and efficacy. Results: Of earlier experience using PKC412 100 mg po bid were reported previously (Colles et al., ASH 2005). This is an updated report of 25 patients treated with PKC412 at a reduced dose of 50 mg po bid given on day 8-21 (arm 1) or day 1-7, 15-21 (arm 2), plus DA and HD-ARAC. Eight out of 23 patients (35%) were FLT3mut, and 15/23 (65%) were FLT3WT as determined by D-HPLC. Results. None of the 19 patients evaluated for safety had drug-related death; the most common non-hematologic toxicities were transient elevations of glucose (16%), AST (16%), bilirubin (11%), ALT (11%), and decreases in potassium (21%), phosphate (11%) and calcium (11%); no grade 3 or 4 nausea, vomiting or pleural effusion were recorded. Twenty-three patients were evaluable for response: 9/12 (75%) achieved CR in Arm 1 and 9/11 (82%) achieved CR in Arm 2. Seven out of 11 (63%) FLT3mut patients and 11/15 (73%) FLT3WT patients achieved CR. FLT3mut patients achieved CR at a significantly higher rate (p=0.047) than FLT3WT patients.
regimen administered to FLT3mut patients in comparison to FLT3WT patients. Accrual to this trial was recently completed (N=40) and updated information on safety and CR rates will be presented. Conclusions. PKC412 at 50 mg po bid given either sequentially or concurrently in combination with DA and HD-ARAC can be given safely with good tolerability in newly diagnosed patients with FLT3mut and FLT3WT AML < 60 years old. This combination merits further study in a randomized fashion (± PKC412) particularly in patients with FLT3mut AML.

**0978**

**ARSENIC TRIOXIDE (ATO) IS SAFE AND EFFECTIVE IN COMBINATION WITH LOW-DOSE ARA-C (LDAC) FOR THE TREATMENT OF ADVANCED MYELODYSPLASTIC SYNDROME (MDS) AND POOR-PROGNOSIS ACUTE MYELOID LEUKEMIA (AML) IN ELDERLY PATIENTS**


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Background/Aims. Treatment outcomes for advanced MDS and elderly AML patients are generally poor. LDAC in elderly AML patients results in a CR rate of approximately 20% and possibly improved morbidity and mortality compared to conventional chemotherapy or supportive care. In MDS patients, the CR rates with LDAC are lower (10-20%) with short duration and no clear benefit over supportive care. On the basis of preclinical data suggesting a possible anti-angiogenic effect of ATO, as well as clinical data showing activity of ATO in MDS, a phase I/II study of ATO in combination with LDAC was initiated in IPSS Int-2/high risk MDS and newly-diagnosed, poor-prognosis AML patients. Methods. ATO was given at a dose of 0.25 mg/kg for days 1-5 and 8-12. LDAC was dose-escalated from 5 mg/m² SC BID to the target phase II dose of 10 mg/m² SC BID for days 1-14 (one treatment cycle). Patients who achieved CR after one treatment cycle were given a second, identical cycle, followed by maintenance treatment of 5 days of LDAC and 2 days of ATO every 28 days. Patients who did not achieve CR after one cycle were given a second cycle beginning between days 21-28, with the addition of ascorbic acid 1g IV within 30 minutes of the ATO infusion. Results. Eighty-three patients have been enrolled to date, 52 with AML and 31 with MDS. A total of 75 patients (49 AML, 26 MDS) are evaluable for response, 69 (46 AML, 23 MDS) of whom were treated with the target dose of LDAC. There were no responses in the 6 patients treated with less than the target dose. Clinical characteristics of the 46 evaluable AML patients treated at the target dose include: mean age 73 yrs (range 55-85 yrs; one patient < 60 yrs with AML and multiple medical comorbidities was included); abnormal cytogenetics 29 (66%); antecedent hematologic disorder 29 (63%); secondary disease 7 (15%). CR was achieved in 17 patients (37%) and CRp in 1 patient, for an overall response rate of 39%, with follow-up 1-11+ mos. Eight patients (44%) required 2 treatment cycles to achieve CR/CRp. Of the 46 AML patients, 5 died prior to day 50 (induction mortality = 11%), 3 (7%) of progressive disease and 2 (4%) of neutropenic sepsis. Clinical characteristics of the 25 evaluable MDS patients include: mean age 70 yrs (range 56-84 yrs); abnormal cytogenetics 17 (81%); prior therapy with 5-azacytide 3 (13%). CR was achieved in 6 (26%) patients, follow-up 3-9+ mos. Three patients (13%) required 2 treatment cycles to achieve CR; there was 1 induction death (4%). Summary. The regimen was generally well-tolerated, with minimal grade 3/4 non-hematologic toxicity and no significant nausea, emesis, diarrhea or mucositis. Alopecia was not seen. Grade 4 hematologic toxicity was observed in all patients. Fluid retention occurred in 56/69 (81%) of patients. There were no clinically significant drug-related arrhythmias. The CR rate in AML was comparable to conventional chemotherapy, with improved tolerability and induction mortality; further investigation is warranted.

**0979**

**CYTOSTATIC RESPONSES TO THE HYPOMETHYLATING AGENT, DECITABINE (DAC), IN A PHASE III TRIAL OF DAC VS SUPPORTIVE CARE (SC) IN PATIENTS (PTS) WITH MYELODYSPLASTIC SYNDROMES (MDS)**

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Backgrounds. Clonal cytogenetic abnormalities are detected in 40%-70% of cases of de novo MDS and 95% of cases of therapy-related MDS, and the incidence increases with poor risk. DAC (DacogenTM) is a cytosine analog that reverses aberrant DNA methylation, leading to re-expression of silenced tumor suppressor genes. Aims. In this analysis, we asked whether the hypomethylating agent DAC leads to cytogenetic response in MDS. Methods. We report cytogenetic response data from a Phase III randomized, open-label trial of DAC vs SC in 170 MDS pts. Eligibility requirements included confirmed MDS (de novo or secondary) fitting any of the recognized French-American-British classifications and an International Prognostic Scoring System (IPSS) score of 0.5 or more as determined by complete blood count, cytogenetics, and bone marrow assessment. Cytogenetics was assessed as a secondary endpoint, whereas primary endpoints were response rate (CR+PR) and time to AML or death. For pts with clonal abnormalities at baseline, follow-up cytogenetic evaluations at study end were available for 26 pts in the DAC arm and 21 pts in the SC alone arm. Results. As previously reported, overall response rate according to International Working Group MDS criteria was 17% (15/89) for DAC vs 0% for SC (p<0.001). Responses occurred in all IPSS groups and were also seen in pts with 5q and 7 deletions. Response rate was 13% (2/16) in pts with 5q deletions and 21% (4/19) in pts with 7 deletions. In pts without 5q or 7 deletions, response rates were 16% (11/67) and 14% (9/64), respectively. Complete cytogenetic responses were observed in 35% (9/26) of DAC pts vs 10% (2/21) of SC pts (p=0.08, Fisher’s exact). Also, 1 pt receiving DAC had a minor cytogenetic response. 10/10 DAC pts with cytogenetic response had clinical benefit (6 CR, 2 PR, 1 hematologic improvement, and 1 with normalization of marrow blast count). The primary toxicity was myelosuppression. Conclusion: DAC induces a substantial rate of cytogenetic responses in pts with MDS, suggesting that the clinical improvements induced by this agent are related to elimination of the neoplastic clone rather than to pure differentiation effects.
Acute lymphoblastic leukemia

0980

SINGLE NUCLEOTIDE POLYMORPHISMS OF THE MTHFR (C677T), MTRR (A66G) AND VITAMIN D RECEPTOR (CD2X-2/GATA) GENES ARE IMPORTANT DETERMINANTS OF OSTEOROPOSIS IN PEDIATRIC ALL PATIENTS

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Background. Corticosteroids and methotrexate have adverse effects on growth and bone mineralisation. Aim and methods. The influence of single nucleotide polymorphism's (SNPs) in the vitamin D receptor gene (VDR; 5'Tdx-2/GATA and 5'BsmI, ApaI, TaqI), methylenetetetrahydrofolate reductase gene (MTHFR, C677T and A1298C), methionine synthase reductase gene (MTRR; A66G), estrogen receptor gene (ER; PvuII/Xbal), glucocorticoid receptor gene (GR; Bcl1) and collagen type I gene (COLIA1; SpI binding site), on bone mineral (applied treatment. In contrast to these carriers of the MTHFR 677T allele had a lower BMD-TB as compared to non-carriers of the MTHFR 677T allele. Carriers of the MTRR 66 G-allele had a lower total body BMD during therapy as compared to non-carriers (MTHFR 677T and MTRR 66G showed a decreased BMD-TB during treatment. Carriers of haplotype 8 of the VDR 5'Tdx-2/GATA polymorphism had a lower BMD-LS and/or BMD compared with non-carriers after 32 weeks (DSDS 0.92, p<0.01) and 1 year of therapy (DSDS 0.95, p<0.01). The MTHFR 1298 C-SNP did not effect BMD values. Carriers of the MTRR 66 G-allele had a lower total body BMD during therapy as compared to non-carriers (MTHFR 677T and MTRR 66G showed a decreased BMD-TB during treatment. Carriers of haplotype 8 of the VDR 5'Tdx-2/GATA polymorphism had a lower BMD-LS and/or BMD compared with non-carriers after 32 weeks (DSDS 0.61, p<0.05 and DSDS 0.69, p<0.05), 1 year (DSDS 0.70, p<0.05 (BMD-LS)), 2 years of therapy (DSDS 1.15, p<0.01 and DSDS 1.18, p<0.01) and 1 year after cessation of therapy (DSDS 0.74, p<0.05 (BMDAD)). Conclusion. No correlations were found between fracture risk and genotype. We identified the MTHFR C677T, MTRR A66G and VDR 5'Tdx-2/GATA SNP's as determinants of treatment-related osteoporosis in pediatric patients with ALL.

0982

LATE RELAPSES IN T-ALL PATIENTS TRUE DISEASE RECURRENCE OR SECOND T-ALL?

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The vast majority of relapses in T-cell acute lymphoblastic leukemia (T-ALL) patients occur relatively early, usually within 2 years from diagnosis, frequently during maintenance treatment. Our previous comparative molecular analyses between diagnosis and relapse of such ‘classical’ T-ALL (26 patients) showed totally (62%) or at least partly (38%) identical T-cell receptor (TCR) gene rearrangement patterns at both disease phases. These results confirm that the relapse clone in these patients originated from the original diagnosis clone, which became resistant to the applied treatment. In contrast to these classical T-ALL, two patients experienced very late T-ALL recurrences (6 and 10 years from diagnosis, respectively) and both patients displayed completely different TCR gene rearrangement sequences between diagnosis and relapse. We hypothesized that such late relapses of T-ALL in fact might represent second malignancies and that patients developing such second leukemias might be genetically predisposed for T-ALL development. We succeeded to investigate 13 T-ALL patients with late relapses, i.e. at least 2.5 years from initial diagnosis. The studies at the DNA level involved detailed comparison of TCR gene rearrangements between diagnosis and relapse (PCR-heteroduplex, sequencing and/or Southern blot analyses) and the detection of gene fusions involving the TAL1 gene and/or TCR genes. We found the evidence of a common clonal origin between diagnosis and relapse in 8 of the 13 patients. In one case, the T-ALL had no clonal TCR rearrangements neither at diagnosis nor at relapse. Finally, in four patients TCR gene rearrangement sequences had completely changed between diagnosis and relapse, suggesting a second T-ALL rather than a relapse. We conclude that approximately 25% of late T-ALL ‘relapses’ in fact represent second malignancies. We are currently planning further genomic analyses to identify common genetic events or common genomic features which might be related to predisposition for development of T-ALL.
ETV6/RUNX1 DIRECTLY DYSREGULATES GENES WITH RUNX1 BINDING SITE VIA MECHANISM REVERSIBLE BY HISTONE DEACETYLASE INHIBITORS

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RUNX1 is implicated in over 30 different translocations in human acute leukemia. RUNX1, can either activate or repress transcription of key regulators of cell growth and differentiation through binding to promoters or enhancer elements. The ETV6/RUNX1 chromosomal translocation is the most common chromosomal aberration in pediatric cancers (25% of ALL). The ETV6 part of the fusion protein contains domains interacting with the mSin3, N-CoR and HDAC-3 corepressors. A part of the RUNX1 gene involved in the fusion carries DNA-binding domain. RUNX1 regulates haematopoietic myeloid cell differentiation and transcriptional activation but the role in lymphoid development is not yet fully understood. We hypothesize that ETV6/RUNX1 causes pathological differentiation block in lymphoid cells. In the current project, we utilized treatment with histone deacetylase inhibitors (HDACi). We have previously confirmed specific effect of HDACi (valproate-VPA, Trichostatin A-TSA) on ETV6/RUNX1 leukemia cells in comparison with lymphoblastic leukemias with different mechanism of leukemogenesis (BCR/ABL and PDGFRα/ETV6). To prove the direct effect of HDACi on ETV6/RUNX1 in vitro, we utilized a target gene of RUNX1, granzyme B (GZMB). To determine whether ETV6/RUNX1 represses GZMB via direct interaction with RUNX1-binding site at GZMB promoter, luciferase activity was measured in HeLa cells transfected with pcDNA3.1-ETV6/RUNX1Myc and compared with HeLa with pcDNA3.1 empty vector. Cells were transfected with pGZMB-luc or pGL3-basic to normalize the luciferase activity(pGZMB-luc/pGL3-basic). Fold change of ~3 FRU indicated that GZMB was downregulated by ETV6/RUNX1. To test the direct effect of HDACi on ETV6/RUNX1, after incubation of HeLa cells with VPA and TSA, luciferase activity was monitored again. Repression activity was reduced in treated transfected HeLa cells to 53% after VPA administration and 49% after TSA administration when compared to untreated cells. We used effect of HDACi on ETV6/RUNX1 leukemia cells and identified ETV6/RUNX1 target genes in lymphoid cells. Analysis of expression profiling of treated (VPA, TSA) vs untreated (control) ETV6/RUNX1[+]+ REH cells showed genes with significantly changed expression after HDACi treatment. This group of genes was compared with a group of genes associated with ETV6/RUNX1 phenotype selected by meta-analysis of expression data of ALL patients. Microarray data of selected genes showed downregulation of JunD, ACK1, PDGFRB in ETV6/RUNX1[+]+ patients as well as in our cell line model with increased expression after HDACi treatment. TCF4 gene was upregulated in the studied group and the administration of HDACi lead to its downregulation. Expression levels of chosen genes were validated by qRT-PCR. JunD - TSA p=0.018, VPA p=0.0008, PDGFRB - TSA p=0.0001, VPA p=0.016, TCF4 - TSA p=0.0001, VPA p=0.0002, ACK1 - VPA p=0.07. Selected genes have a fundamental role in cell proliferation and cell cycle progression therefore their role in leukemogenesis is presumptive. We show for the first time direct transcription repression by ETV6/RUNX1 on GZMB gene model. These data also support our hypothesis that HDACi affect ETV6/RUNX1[+] cells via direct interaction with ETV6/RUNX1 protein, and that treatment with HDACi may release pathological differentiation block caused by ETV6/RUNX1 aberrant transcription factor.

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MINIMAL RESIDUAL DISEASE STATUS IS THE MOST IMPORTANT PREDICTIVE FACTOR IN ADULTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA. PALG 4-2002 PROSPECTIVE ALL-MRD STUDY

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Current therapeutic protocols for adult acute lymphoblastic leukemia (ALL) take into account the risk of relapse, in order adjust the treatment intensity to individual patient needs. It is postulated that in addition to classical risk criteria including age, cytogenetics, immunophenotype, and tumor burden, also minimal residual disease (MRD) should be considered for treatment decisions. The aim of this prospective study was to evaluate the feasibility and prognostic significance of MRD detected with the use of immunophenotyping for disease-free survival (DFS) of ALL patients treated according to 4-2002 protocol of the Polish Adult Leukemia Group (PALG). Induction therapy included prednisolone, asparaginase and 4x etoposide + vincristine. Consolidation consisted of 2x high-dose AraC + cyclophosphamide, 2x methotrexate + etoposide, mercaptopurine, and CNS prophylaxis including irradiation. Patients stratified to high risk group according ‘classical’ criteria based on those formerly developed by GMALL (bcr/abl+, WBC>30 G/L, preB or preT antigen) were treated with maintenance for two years. MRD was tested at the level of 0.1% after completion of induction and consolidation therapy in patients achieving CR, employing multicolor flow-cytometry. For patients with specific antigen combinations a standard quadtrans method was used, for the remaining ones we applied a new empty spaces method taking into account an individual antigen expression on blast cells. The forbidden gates were established with the use of triple staining by comparison with the pattern obtained for healthy volunteer bone marrow donors. At least two antigen combinations were tested for each patient. One-hundred ten ALL patients (B-lineage 82%, T-lineage 17%), aged 50 years (17-61) treated in 16 hematological centers were included in the analysis. CR rate equaled 80%. Among patients who achieved CR, 24% were assigned to standard risk group, 76% - to high risk group, according to classical criteria. MRD evaluation was possible in all CR patients. In 50% of patients MRD was negative after both induction and consolidation - MRD(−) group, whereas in the remaining 50% of cases MRD was detected at least once - MRD(+) group. At 3 years the probability of DFS in MRD(−) and MRD(+) group equaled 58% and 28%, respectively (p=0.04). The prognostic value of MRD status for DFS was more pronounced in patients with standard risk ALL: 80% for MRD(−) vs. 0% for MRD(+) (p=0.048), than in those with high risk ALL: 51% vs. 33%, respectively (p=0.25). In a multivariate analysis including classical prognostic criteria the MRD status remained the only significant predictive factor (HR: 1.33 (1.24-22.46), p=0.04). We conclude that immunophenotyping employing empty spaces method is feasible for MRD evaluation in adults with ALL. MRD status after induction and consolidation is the most important predictive factor for DFS. In particular, patients assigned to standard risk according to classical criteria can be further stratified and those with MRD detected after induction and/or consolidation should be offered intensified treatment with the use of hematopoietic cell transplantation.
Hodgkin's Lymphoma - Clinical Trials

0985
RECENT INTERIM ANALYSIS OF THE HD11 TRIAL OF THE GHSG: INTENSIFICATION OF CHEMOTHERAPY AND REDUCTION OF RADIATION DOSE IN EARLY UNFAVOURABLE STAGE HDGNN'S LYMPHOMA

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Backgrounds. Combined modality treatment consisting of chemotherapy (CT) followed by involved field radiotherapy (IF-RT) is the standard treatment for early unfavourable Hodgkin's lymphoma (HL). Despite high complete remission (CR) rates, failures are common. We thus compared the baseline-dose BEACOPP regimen with ABVD and 20 Gy IF-RT in a prospectively randomized trial (HD11) in an attempt to improve outcome in this group of patients. Methods. Between May 1998 and January 2003, 1570 patients (pts) aged 16-75 with untreated intermediate stage HL (CS I, II A with risk factors or IIB with elevated ESR and/or ed intermediate stage HL (CS I, IIA with risk factors or IIB with elevated ESR and/or ed intermediate stage HL (CS I, IIA with risk factors or IIB with elevated ESR and/or ed intermediate stage HL (CS I, IIA with risk factors or IIB with elevated ESR) were randomized according to a factorial design between 4 cycles of ABVD followed by 30 Gy IF-RT (arm A - standard treatment), 4 ABVD + 20 Gy IF-RT (arm B), 4 baseline-dose BEACOPP + 30 Gy IF-RT (arm C) and 4 baseline-dose BEACOPP + 20 Gy IF-RT (arm D). Results. In the fifth preplanned interim analysis, 1298 pts were evaluable for the chemotherapy comparison and 1274 for the radiotherapy comparison. Patient characteristics were well balanced between the treatment arms. 95% of patients treated reached CR, 2% had progressive disease, 8% relapsed and the total mortality rate was 4% with no significant differences between treatment arms for either endpoint. The most frequent haematological toxicities during chemotherapy were leucopenia observed in 52% of pts (ABVD 25%, BEACOPP 58%) and anaemia in 4% of pts (ABVD <1%, BEACOPP 7%). Infection rate was 5% (ABVD 3%, BEACOPP 7%). The most frequent toxicity during radiotherapy was dysphagia in 5%. 14 secondary neoplasias were observed: 2 AML, 4 NHL, 8 solid tumors with no significant differences between treatment arms. After a median observation time of three years, freedom from treatment failure (FFTF) was 87% (95%-CI 85-89) and overall survival (OS) was 96% (95%-CI 95-97). Both for FFTF and OS, there was no sequential significant difference either between ABVD (FFTF 87%, OS 97%) and BEACOPP (FFTF 88%, OS 96%) nor 30 Gy (FFTF 90%, OS 97%) and 20 Gy IF-RT (FFTF 87%, OS 97%). Conclusions. At three years of median observation time, no sequential significant differences in treatment outcome were detected, neither between chemotherapy regimens nor between the different doses of radiotherapy, despite more relapses in 20 Gy radiotherapy arms.

0986
COMBINED MODALITY TREATMENT OF TWO OR FOUR CYCLES OF ABVD FOLLOWED BY INVOLVED FIELD RADIOTHERAPY IN THE TREATMENT OF PATIENTS WITH EARLY STAGE HODGKIN'S LYMPHOMA: UPDATE INTERIM ANALYSIS OF THE RANDOMISED HD10 STUDY OF THE GERMAN HDGNN'S LYMPHOMA GRO

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Background and Aim. Combined modality treatment is regarded as standard by most study groups for patients with early-stage Hodgkin's lymphoma (HL). However, the optimal chemotherapy, the number of cycles needed and the optimal radiotherapy dose is still unclear. The GHSG thus conducted a randomised study for patients with early stage favourable Hodgkin's lymphoma (HD10) in which these questions were addressed. Methods. A total of 1370 patients were randomised from May 2000 to April 2003 between two or four cycles of ABVD and independently to 20 Gy or 30 Gy involved field (IF) radiotherapy. Results. For the second interim analysis at a median follow up of 28 months, 847 patients were evaluable. Patients were equally balanced for age, gender, stage, histology, performance status and risk factors. Compared with two cycles, there was more toxicity in patients receiving four cycles of ABVD for leucopenia, hair loss and infection. Concerning radiotherapy dose, there was more toxicity associated with 30 Gy for dysphagia, mucositis and leucopenia. The rate of complete remissions ranged between 98% and 99% with no significant differences among treatment arms. Freedom from treatment failure (FFTF) and overall survival showed no differences between the four treatment arms. The curves for overall survival and FFTF were nearly superimposable for all four arms. Conclusion. This analysis suggests that 2 chemotherapy cycles with involved field radiotherapy may be sufficient for patients with early favourable HL, but a reliable assessment must await the final analysis including all randomised patients and with adequate follow-up. The results of the third interim analysis (10/2005) included 10 patients with a median follow up of more than 3 years will be presented.

0987
PREDICTIVE VALUE ON TREATMENT OUTCOME OF EARLY FDG PET SCAN IN ADVANCED STAGE HODGKIN'S DISEASE TREATED WITH CONVENTIONAL CHEMOTHERAPY IS SUPERIOR TO IPS SCORE

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Backgrounds. FDG-PET scan performed early during therapy (ChT) is a powerful prognostic tool in lymphomas. Aims. Starting in January 2002, 152 new, advanced-stage HD pts, consecutively admitted to twelve Italian hematological institutions were enrolled in a prospective multicenter clinical trial aimed at comparing the predictive value on treatment outcome of International Prognostic Score (IPS) with FDG-PET scan performed after two courses of ABVD in untreated advanced stage HD patients (pts). Patients. The mean age was 33.6 years (14-79), the male to female ratio 65/67; advanced disease (stages IIb-IVb) was present in 94, and stage IIA with adverse prognostic factor (> 3 nodal sites involved, sub-diaphagmatic presentation, bulky disease and ESR > 40) in 38. Bulky and extra-nodal disease were recorded in 47 and 40 pts, respectively. All pts were staged at baseline, after 2 courses of ChT and at the end of treatment by CT scan and FDG-PET scan (CT0, PET-0; CT-2, PET-2 and CT-6, PET-6, respectively). The mean interval between the end of the second ChT course and PET-2 was 11.6 days (2-32); the interval between the end of the therapy (including radiotherapy) and PET-6 was never shorter than 50 days. 126/132 pts. were treated with ABVD x 6; 6 by COPP/EBV/CAD x 6. At the end of ChT in 66/132 pts. with bulky disease consolidation radiotherapy was given. All patients were given the therapy programmed at baseline, except in case of overt progression. Results. The mean follow-ups from the diagnosis and from final resting were 609 days (73-1513) and 402 days (0-1240), respectively. 108 pts attained CR while 24 were chemoresistant: 19 showed disease progression during therapy I was RI and 4 showed early relapse (within 6 months) after CR entry: (+28 - +178 days). One out of the 108 pts attaining CR showed a late relapse 16 months after CR entry. In univariate analysis, the T4N2B 1 pet-2 < 0.01, the clinical factors that were significantly associated with a higher probability of treatment failure were stage (p < 0.01), International Prognostic Score (p < 0.01), WBC (p < 0.01), Extra-nodal sites (p < 0.01). The only factor independently significant for relapse/progression probability in multivariate analysis was PET-2, with a very high hazard ratio (0.98 95% CI 1.79 - 207.0). In terms of treatment failure, the Positive Predictive Value (PPV) of a PET-2 and IPS (Score 0-2 vs 3 or more) were 88% and 41% and the Negative Predictive Value (NPV) were 96% and 88%, respectively. The sensitivity of PET-2 and IPS were 92% and 46%, the specificity were 97% and 85% and the overall accuracy 96% and 78%, respectively. The 2-yr FFS and FFS probability for PET-2 negative and for PET-2 positive patients were 97% and 97% and 7% and 18%, respectively (FFS Log Rank test = 135.1, p < 0.01; FFS log rank test=114.0, p<0.01). Conclusions. PET-2 scan is the most powerful tool so far available for predicting treatment outcome in advanced-stage HD.
**0988**

**AMH AND INHIBIN B ARE VALUABLE NEW MARKERS FOR GONADAL DAMAGE AFTER THE TREATMENT OF M.HODGKIN WITHOUT RADIOTHERAPY**


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**Background.** An important long-term effect of both radiotherapy and chemotherapy is gonadal dysfunction. Aim of this study is to evaluate the gonadal long-term effects of the treatment for childhood M. Hodgkin (HD) with combination chemotherapy (ABVD or EBVD with/without MOPP) and to identify markers for long-term follow-up of gonadal function. Methods. Eighty-six pediatric HD patients treated from 1974-1998 were included. All patients were in complete remission. Median follow-up was 15.5 yr. (range 5.6-30.2 yr.), median age at follow-up was 27.0 yr. (range 17.7-42.6 yr.). Follicle stimulating hormone (FSH), luteinizing hormone (LH) and inhibin B were determined in all patients. Additionally, in men testosterone and sex hormone binding globuline (SHBG) and in women 17 βestradiol and anti-Müllerian hormone (AMH) were determined. In 20 men semenanalyses were performed. Results. In men treated with MOPP median FSH (16.6 U/l vs. 2.4 U/l; p<0.001) and LH (5.7 U/l vs. 2.5 U/l; p<0.001) were significantly increased as compared to patients treated without MOPP. Inhibin B (17.5 ng/l vs. 145 ng/l; p<0.001) and semen concentration (1.1*10^6/mL vs. 49.5*10^6/mL; p<0.05) were significantly decreased. Inhibin B was strongly correlated with semen concentration (rs=0.83; p<0.001). FSH (rs=0.68; p<0.001) and inhibin B (rs=0.68; p<0.001) were correlated with cumulative dose procarbazine. In women no significant differences in LH, FSH, inhibin B or estradiol between patients treated with or without MOPP were found, but AMH was significantly lower in patients treated with MOPP as compared to patients treated without MOPP (0.39 µg/L vs. 1.40 µg/L; p<0.001). AMH levels were correlated with cumulative dose procarbazine (rs=-0.54; p<0.01). Conclusion. This study shows that AMH and inhibin B are valuable new serum markers for gonadal damage after pediatric HD. In men inhibin B is strongly correlated with semen concentration, whereas in women AMH detects early gonadal damage even in cases with normal LH/FSH levels.

**0989**

**THE POLYMORPHISM IN THE INTERLEUKIN-10 GENE PROMOTER AT -592 IS A PROGNOSTIC MARKER IN HODGKIN’S LYMPHOMA**


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**Backgrounds.** Hodgkin’s lymphoma is characterized by an abundant immune infiltrate surrounding the malignant Reed-Sternberg cells, and it is thought that the production of cytokines contributes to this abnormal immune response. Single nucleotide polymorphism in the IL-10 promoter region of cytokine genes are key factors for cytokine production and may modify the biology of the disease. Recently, differences in the prognosis according to the Interleukin-10 (IL-10) genotype have been shown in patients with diffuse large B cell lymphomas (Lech-Mariana et al, Blood 2004; 103:3529). Aim. To assess the role of polymorphisms in the Interleukin-10 gene on progression-free survival in Hodgkin’s lymphoma. Methods. We assessed the distribution of frequencies of polymorphic allele variants in the IL-10 gene (T-5875A; G-2849A, C-2763A, A-1082G and C-592A) in 204 patients with Hodgkin’s lymphoma and analysed for associations with patient characteristics and prognosis. The polymorphism were analyzed using a multiplex amplification and mismatched polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP). DNA was extracted either from peripheral blood or paraffin-embedded lymph node biopsies from 204 patients with Hodgkin’s lymphoma (median age 52 years, range 14-77 years; 91 females and 113 males). 194 patients were treated with standard chemotherapy regimens. 115 patients received ABVD, 34 pts a modified Stanford V regimen (substituting 6 mg/m² metchloramine with 650 mg/m² cyclophosphamide), 24 pts MOPP (+ABVD), 21 pts BEACOPP. The prognostic role of allelic variants were analyzed as SNPs, and of haplotypes which were reconstructed using the PHASE programme. Results. The distribution of allele frequencies in Hodgkin’s lymphoma at position -592 of the IL-10 gene was as follows: 46% were homozygous for the CC genotype, 40% were heterozygous and 14% were homozygous for the AA genotype. The IL10 -592AA genotype was associated with a decreased progression-free survival (p=0.007). The probability of progression-free survival at a median time of observation of 4 years for patients homozygous for the IL-10 -592 AA genotype was 35% (95% C.I. 14-54%), while for heterozygous patients and for patients homozygous for the -592 C allele it was 70 and 74% (95% C.I., 56 -80, and 61-85), respectively. When the analysis was restricted to 115 patients treated with ABVD chemotherapy, essentially the same differences in progression-free survival were observed. In univariate analysis of established prognostic factors, stage proved to be of prognostic value in our patient group (limited disease in stage I-IIA vs advanced disease in stage IIb-Iv, p=0.013). The Cox multivariate analysis showed that IL-10 -592AA genotype and stage were independent prognostic factors (p=0.02 and 0.016, respectively). Conclusion. Our study indicates that the IL-10 genotype can predict clinical outcome in patients with Hodgkin’s lymphoma and points to the importance of the genetic background of the host.
CD95L EXPRESSING ANTIGEN-PRESENTING CELLS PREVENT ANTIGEN-SPECIFIC T CELL RESPONSE BY APOPTOSIS INDUCTION AND INHIBITION OF T CELL ACTIVATION

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Background. Selective depletion of antigen-specific T cells e.g. through induction of apoptosis via the CD95 system may have widespread applications in transplantation settings or in the treatment of autoimmune diseases. Antigen presenting cells (APC) expressing death-inducing ligands such as CD95 ligand (CD95L) could theoretically be used as immunomodulators in an antigen-specific counterattack system. Since human naive T cells are resistant to CD95-mediated apoptosis and acquire CD95-sensitivity only after activation, CD95L expressing APC might selectively deplete antigen-specific T cells while leaving naive T cells untouched. Aims. We studied the modulation of an alloimmune response and changes in T cell activation in the presence of CD95L expressing APC. Methods. The HLA-A1 expressing lymphoblastoid cell line CIR.A1 was transfected with membrane-bound CD95L (m-CD95L), which was stably expressed on the cell surface of the APC due to a mutation in the metalloproteinase cleavage site. HLA-A1 negative T cells were stimulated with m-CD95L expressing CIR.A1 cells or with a mock transfectant to study the development of the HLA-A1 specific alloimmune response. Results. m-CD95L expressing APC were able to induce apoptosis in CD95 expressing activated primary T cells. Constitutive presence of m-CD95L in the stimulation cultures inhibited the development of CD4+ and CD8+ HLA-A1-specific T cells. However, immunity towards third-party, viral, and bacterial antigens was maintained and T cells spared from depletion could be induced to develop cytotoxicity towards unrelated antigens. Interestingly, inhibition of HLA-A1 specific T cell response absolutely requires the co-expression of m-CD95L and HLA-A1 antigen on the same APC. The simultaneous analysis of proliferation and apoptosis induction in HLA-A1 negative T cells activated with m-CD95L expressing APC indicated that activated T cells are depleted by cell death induction while proliferation of naive T cells was inhibited. naive T cells activated by m-CD95L expressing APC exhibited a reduced expression of activation markers (CD25, CD69, CD71, HLA CII) and Th1 and Th2 cytokines. Ca influx was diminished when cells were stimulated by CD95L expressing APC compared to the mock transfectant. However, differences in NF-κB activation were not observed independent whether m-CD95L was absent or present. Efficiency of inhibition of T cell activation by m-CD95L expressing APC was dependent on the expression level of m-CD95L. Conclusions. m-CD95L expressing APC represent efficient immunomodulators to achieve antigen-specific tolerance since they simultaneously induce apoptosis in activated T cells and prevent T cell activation of naive T cells without impairment of immune responses towards unrelated antigens.

THE PROTO-ONCOGENE EVI1 INDUCES FETAL ANEMIA IN A CONDITIONAL TRANSGENIC MOUSE MODEL

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Background. Absent expression of the proto-oncogene EVI1 has been observed in patients with acute myeloid leukemia, chronic myeloid leukemia or myelodysplastic syndrome carrying 3q26 aberrations. Patients with high EVI1 expression respond poorly to anti-leukemic therapy. Although it is generally believed that EVI1 transforms hematopoietic stem cells, there is evidence that EVI1 may interfere more directly with myeloid or erythroid development, and lineage-specific effects may play a role in the pathogenesis of leukemia or myelodysplastic syndromes. Aims. Since MDS with 3q26 abnormalities and aberrant EVI1 expression is characterized by severe anemia, our aim was to determine the direct effects of EVI1 on erythropoiesis in vivo. Moreover, our approach allowed us to investigate the effects of EVI1 when expressed at different stages of erythroid differentiation. Methods. To prevent the embryonic lethality associated with conventional EVI1 transgenic models and to allow the study of effects of EVI1 in separate hematopoietic lineages, we established transgenic mouse lines with conditional, Cre- inducible hematopoietic expression of EVI1. In these Vav-LSL-EVI1 transgenic lines, EVI1 expression is blocked by a loxP-flanked transcriptional stop sequence (LSL). The EVI1 transgenic lines were crossed with two different erythroid lineage specific Cre transgenic lines to specifically induce EVI1 expression at different stages of erythroid differentiation. The EpOr-Cre and pEV-Cre transgenic lines express Cre from the BFU-E and CFU-E stage onward, respectively. Results. Erythroid-specific EVI1 overexpression induced fetal anemia, with major defects in primitive and definitive erythropoiesis. Fetal livers from both Vav-LSL-EVI1/pEV-Cre and Vav-LSL-EVI1/EpOr-Cre double transgenic animals were small, pale and contained decreased cell numbers as compared to livers from single transgenic or wild type littermates. However, the phenotype in VAV-LSL-EVI1/EpOr-Cre embryos was clearly more severe. Colony assays demonstrated that VAV-LSL-EVI1/EpOr-Cre transgenic fetal livers contained less BFU-E and CFU-E erythroid progenitors, while in VAV-LSL-EVI1/pEV-Cre embryos only CFU-E numbers were reduced. Moreover, a more complete block in terminal erythroid differentiation and a more profound increase in the number of primitive and definitive fetal liver erythroid cells were observed in VAV-LSL-EVI1/EpOr-Cre embryos. Ex vivo experiments suggest that the EVI1-induced embryonic lethality in VAV-LSL-EVI1/EpOr-Cre as opposed to Vav-LSL-EVI1/pEV-Cre mice may be due to a reduced sensitivity of VAV-LSL-EVI1/EpOr-Cre erythroid cells to respond to Epo. Summary/Conclusions. Our data suggest that EVI1 pathetically and prenatailly affects the survival, expansion and differentiation of erythroid progenitors in vivo, and that the severity of the defects increases when EVI1 is induced at an earlier stage of erythropoiesis. We have established a conditional EVI1 transgenic mouse model that in combination with other inducible or lineage specific Cre-lines, e.g. Mx1-Cre can be applied to study the involvement of EVI1 in MDS and AML.

ROLE OF LYMPHOID MICROENVIRONMENT IN INHIBITION OF APOPTOSIS AND ACTIVATION OF PI3-K/AKT PATHWAY AND PTEIN IN B-CELLS

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Background. The accumulation of the malignant B cells in chronic lymphocytic leukemia (B-CLL) appears to be due to inhibition of apoptosis and long survival of the leukemic cells. This could be due to the activation of anti-apoptotic mechanisms in the leukemic cells through their interaction with the lymphoid microenvironment. Aims. The aim of this study is to elucidate the role of the lymphoid microenvironment in activation of the anti-apoptotic PI3-K/Akt pathway by B-CLL. Methods. B-CLL cells were co-cultured with human fibroblasts. Induction of apoptosis was associated with a significant decrease in the intracellular PIP3, PI3-K, PDK1 and Akt1, NF-κB, IKK, and JNK activity. The supportive effect of stromal fibroblasts was abolished in the presence of PI3-K inhibitors (wortmannin or LY294002) or siRNAs against PI3-K and Akt. Results. Pharmacological inhibitors and siRNAs against PI3-K and Akt were applied to explore the anti-apoptotic effect of this pathway in B-CLL. It was found that the supportive effect of stromal fibroblasts was abolished in the presence of PI3-K inhibitors or siRNAs against PI3-K (p110β subunit) and Akt1. These inhibitors significantly reduced the supportive effect of stromal fibroblasts and induced apoptosis in B-CLL cells. Interestingly, the leukemic cells were far more sensitive to PI3-K inhibition than T cells, monocytes and fibroblasts. Induction of apoptosis was associated with a significant decrease in the intracellular PIP3, PI3-K, PDK1 and Akt1, NF-κB, IKK, and dephosphorylation (activation) of tumour suppressor protein PTEN. Studies using phosphospecific anti-PTEN antibody demonstrated that PTEN is involved in inhibition of apoptosis of B-CLL cells and that interaction of the leukemic cells with lymphoid microenvironment maintains the activation of this pathway. The data also suggest that targeting this pathway represents a new therapeutic approach in B-CLL.
Drug resistance and treatment failure in acute leukemia has been attributed to apoptosis resistance in leukemia cells as defects in apoptosis signal transduction are commonly acquired during malignant transformation. However, expression analysis of apoptotic molecules with regard to clinical outcome has so far failed to identify apoptosis defects with prognostic value. Since the efficacy of apoptosis signaling is probably not sufficiently represented by the expression of apoptosis molecules alone, we developed and evaluated different assays to assess the function of apoptotic pathways in primary leukemia cells. Flow cytometric quantification of caspase activation by cleavage of the rhodamine derivative (Z-DEVD)2R revealed a broad variation in the extent of caspase-3 activity in primary pediatric B-precursor ALL cells upon cultivation in medium, which might be of prognostic relevance. Despite similar induction of cell death, a differential activation of caspase-3 by Cytarabine and Cyclophosphamide could be assessed, indicating drug specific differences in activation of apoptosis signaling. In a xenotransplant disease model for pediatric ALL, drug induced caspase activation could be quantified, demonstrating its potential use for monitoring drug efficacy in vivo. In order to test the functional integrity of a core apoptosis signaling pathway, we have developed and evaluated a method for the simultaneous measurement of two apoptogenic events in individual cells: caspase-3 activation and cytochrome c release, using conformation sensitive monoclonal antibodies. This method proved to identify deficient mitochondrial apoptosis signaling in leukemia cells overexpressing Bcl-2 by a pattern of apoptosis resistance, deficient cytochrome c reduction and partial processing of caspase-3. By combination of these techniques, we were able to analyze and, more importantly, to quantify potential defects in apoptosis signal transduction on a single cell level in patient samples cultured in vitro. We analyzed the activation of apoptosis signaling in primary leukemia cells during apoptosis induction by the physiologic stimulus of lack of survival factors in order to identify constitutive defects in apoptosis signaling in individual leukemia samples. Activation and mutual correlation of cytochrome c release and caspase-3 activation was quantified in 78 patient samples of precursor B-cell ALL. We identified a novel parameter, CRAC (Cytocrome c - Related Activation of Caspases 3) reflecting proficient or deficient cytochrome c related caspase activation in the individual patient sample with prognostic impact on treatment failure and relapse. At a median follow-up of 31 months, disease-free survival was 84 months (95% CI = 76 to 91 months) and 66 months (95% CI = 52 to 80 months) for patients with positive and negative CRAC respectively (p=0.019). CRAC may thus serve as a functionally defined risk factor for treatment stratification. Functional analysis of apoptosis signaling in primary leukemia may help to identify molecular targets for improvement of anti-leukemic treatment.
Anemia/Red blood cells

0995 COLD SHOCK DOMAIN PROTEIN A (CSDA) ACTS AS REPRESSOR FACTOR OF γ GLOBIN GENE EXPRESSION IN VIVO

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Impaired hemoglobin switching leading to persistent expression of fetal globin genes in adults (HPFH) offers great therapeutic potential for hemoglobinopathies and much effort is underway to clarify the molecular basis of this mechanism. In order to identify and study regulatory factors putatively involved in γ-globin gene expression, we examined the reticulocyte mRNAs differently expressed in three siblings presenting different levels of HbF and varied severity of β-thalassemia intermedia conditions, even though sharing the same α- and β-globin gene cluster genotypes. In fact, all of them showed the homozygous state for the β+ IVS1-6 (CT) mutation associated to haplotype VI chromosomes and a normal set of α-globin genes. To investigate the possible causes of the variations in γ-globin gene expression, extensive sequence analysis was performed on putative regulatory regions within the β-globin gene cluster. Results showed the same genetic background in all the siblings and excluded HPFH mutations. It was thus supposed that genetic determinants not linked to the β-globin gene cluster were responsible of the different γ-globin gene expression levels. To explore this hypothesis, the reticulocyte transcriptome was analyzed by a differential mRNA display approach, revealing several bands differentially displayed in the sample from the brother respect to his sisters. Selected bands were cloned and sequenced. A complete homology (greater than 95%) with the cDNA sequence of the cold shock domain protein A (CSDA) acting as repressor factor for several hematopoietic genes and previously reported to be able to interact with the γ-globin gene promoter was found for two of the clones originated from bands with increased expression in the brother. Quantitative real time PCR analysis of CSDA and γ-globin gene mRNA levels was performed on reticulocyte RNAs to confirm data obtained by differential display and revealed an inverted correlation between HbF values and CSDA mRNA levels, comparable to that found between CSDA and γ-globin gene mRNAs. To analyze the role played by CSDA in regulating the expression of γ-globin genes, transient RNAi was used to elicit its knockdown in K562 cell line. Results showed a two-fold increased level of γ-globin mRNA when CSDA expression was interfered at about 40-50%. CSDA has been previously reported to interact with the -200 promoter region of the γ-globin gene where some HPFH mutations fall and a possible mechanism of trans-acting regulation of γ-globin gene expression has been proposed. Our data, in agreement with this hypothesis, provide further insights into the involvement of CSDA in the control of γ-globin genes expression. In fact, in our case, no HPFH mutations were detected but it is rather conceivable, on the basis of RNAi results, that a quantitative defect of CSDA expression may produce a significant persistence of HbF in adult life, thus suggesting possible novel targets for gene therapy in hemoglobinopathies.

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0996 ADJUVANT INTRAVENOUS THERAPY POTENTIATES EPOETIN β TREATMENT IN ANEMIC, NON-IRON-DEPLETED PATIENTS WITH LYMPHOPROLIFERATIVE DISORDERS: RESULTS OF THE NIFE STUDY

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Backgrounds. Anemia is a common complication of malignancy. Inflammatory cytokines reduce erythropoietin production and cause disturbances in iron metabolism, most notably impaired iron uptake and mobilization from iron stores. This situation, named functional iron deficiency, may be one reason why only ~60% of cancer patients respond to epoetin therapy. Previous studies reporting a potentiating effect of intravenous (IV) iron to epoetin therapy in cancer patients may not have excluded iron depletion as a cause of anemia. AIMS. To assess whether adjuvant IV iron therapy potentiates epoetin β (NeoRecormon™) treatment of anemia in patients with lymphoproliferative disorders (LPD) and proven iron presence in the bone marrow. METHODS. NFe (NeoRecormon with intravenous Iron [Fe]), an open, prospective, randomized study in anemic patients with indolent LPD not receiving chemotherapy, was performed in 15 Swedish centers. Sixty-seven patients with indolent non-Hodgkin’s lymphoma (n=19), chronic lymphocytic leukemia (n=23) or multiple myeloma (n=25) were randomized to receive either epoetin β only or both epoetin β and IV iron. Inclusion criteria were cancer-associated anemia (hemoglobin [Hb] ≥9 to ≤11 g/dl) and demonstration of stainable iron in a bone marrow aspirate. Major exclusion criteria were transfusion dependency, recent chemotherapy, serum ferritin >800 ng/mL or anemia from other causes. Epoetin β 30 000 IU once weekly (QW) was given subcutaneously for 16 consecutive weeks.

Dose adjustments were performed according to the label. Iron sucrose (Venofer™) 100 mg QW IV was given from week 0 to 6, followed by 100 mg every 2 weeks. The primary efficacy parameter was change in Hb level from baseline to end of treatment (EOT). Secondary endpoints were Hb response rates (% of patients with Hb ≥2 g/dl in the absence of red blood cell transfusion), dose of epoetin β and iron kinetics. All 67 randomized patients were included in the intention-to-treat (ITT) population, and 60 completed the study. Three patients received transfusion and/or chemotherapy and were not included in the per-protocol (PP) population of 57 patients. Results. There were no significant differences in key parameters between the two groups at baseline. The epoetin plus-iron group had a significantly higher mean change in Hb level from baseline to EOT than the epoetin-only group (2.76 vs 1.56 g/dl; p<0.0002; ITT population) and 2.91 vs 1.50 g/dl (p<0.0001; PP population). Hb response was reached earlier and in significantly more patients in both the ITT (79% vs 50%; p<0.02) and the PP (93% vs 53%; p=0.001) populations at EOT (Figure). Furthermore, a lower dose of epoetin was required in the group receiving iron compared with the group receiving epoetin alone (p=0.051). Conclusion: Compared with epoetin only, use of concomitant IV iron significantly increased Hb concentrations and the proportion of Hb responders in non-iron-depleted patients with LPD and cancer-associated anemia. Moreover, a lower dose of epoetin was needed to achieve these better and quicker hematopoietic responses.

References
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Backgrounds. Red cell hemostasis is under the control of a highly sensitive negative feedback mechanism in which the glycoprotein hormone...
erythropoietin (Epo) stimulates red cell production. Epo is synthesised by the kidney in response to hypoxia and the hypoxia-inducible factor (HIF) transcription complex, which consists of an α and a β subunit, regulates this process. Although both subunits are constitutively expressed, the α subunit is undetectable at the protein level due to continual targeting to the proteasome. In the presence of oxygen, members of the prolyl hydroxylase domain (PHD) group of enzymes actively hydroxylate proline 402 and 564 in the oxygen dependent degradation (ODD) domain of HIF-1α. Upon hydroxylation the von Hippel Lindau (VHL) protein is able to associate and ubiquitinylation occurs, consequently the α subunit is posttranslationally degraded. Although defects in the VHL gene have been identified in the many familial erythrocytosis cases, which are characterized by red cell hyperplasia and no identified secondary cause, there remains a considerable number of cases where the defect remains elusive. Aim: To screen members of the PHD family of prolyl hydroxylases for molecular defects individuals with erythrocytosis and assess the impact of any mutations detected on the Epo negative feedback pathway. Methods: DNA was prepared and FCR-direct sequencing of the three members of the PHD family was performed. In vitro binding and enzymatic functional assays were performed using in vitro translated wild type and mutant PHD2 protein. Results: A heterozygous change of C to G at base 950 in PHD2 was detected in three erythrocytosis individuals from one family. All affected members exhibited subtly raised haematocrits with inappropriately normal Epo levels. The C950G base change was not detected in 200 normal control samples. This mutation results in loss of proline 317, located 2 amino acids away from an iron chelating residue in the active site, and replacement with arginine. The Pro317Arg mutant was found to exhibit reduced affinity for HIFα and its ability to hydroxylate HIFα was greatly impaired. Summary: In vitro binding and enzymatic assays have established that the Pro317Arg mutation would impair the function of PHD2, resulting in less HIF-1α being hydroxylated and allowing more to escape posttranslational degradation. In addition, the mutation in PHD2 indicates that this is the main prolyl hydroxylase active in the regulation of HIF-1α in the Epo pathway. There is now some evidence to suggest that deletion of PHD2 may play a role in the development of endometrial cancer thus raising the possibility that PHD2 may be analogous to VHL, where impaired function causes erythrocytosis while loss of function results in a cancer syndrome.

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SELECTED IMIDS IMMUNOMODULATORY DRUGS: NEW APPROACHES TO THE REGULATION OF ERYTHROPOIESIS AND HEMOGLOBIN SYNTHESIS IN HEMOGLOBINOPATHIES

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Sickle cell anemia (SCA) and β-thalassemia constitute a public health problem worldwide and new therapies are needed. Inhibitors of hemoglobin S (HbS) polymerization is a major target for therapeutic approaches in SCA. New experimental therapies including hydroxyurea (HU) have attempted to augment the synthesis of fetal hemoglobin (HbF) and improve upon current treatment. Clinical trial results have demonstrated that lenalidomide (Revlimid®), recently approved by the FDA, reduces or even eliminates the need for red blood cell transfusions in some anemic myelodysplastic patients. We have examined whether CC-4047 and lenalidomide, two distinct IMiDs (immunomodulatory drugs), currently under evaluation for the treatment of hematological cancers could regulate erythropoiesis and hemoglobin synthesis. For this purpose, we used an in vitro culture model to differentiate human erythroid progenitors from bone marrow or peripheral blood CD34+ cells. We demonstrate that CC-4047 is a potent inducer of fetal hemoglobin (HbF) and synergize with hydroxyurea (HU) during erythroid differentiation of CD34+ progenitors isolated from healthy and SCA donors. In addition, CC-4047 and lenalidomide modulated erythropoiesis, slowing erythroid maturation and increased proliferation of immature erythroid cells. Unlike other inducers of fetal hemoglobin such as HU, 5-aza-cytidine and butyrate, CC-4047 and lenalidomide were not cytotoxic. Gene expression profiling of erythroid differentiation cells showed that our drug regulate specific erythroid transcription factor genes that participate in hemoglobin synthesis and genes involved in cell cycle and cellular differentiation. CC-4047 controls globin gene expression during erythroid differentiation by inducing sustained expression of fetal and embryonic hemoglobin synthesis. Our results support the hypothesis that CC-4047, alone or in combination with current approved therapies, can restore effective erythropoiesis and increase the ratio of fetal to adult hemoglobin. In addition, CC-4047 has the ability to inhibit TNF-α production and help to limit the inflammatory state in sickle cell patients. In conclusion, CC-4047 may represent an innovative new therapy for β-hemoglobinopathies.

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RESPONSE OF MYOCARDIAL T2* TO ORAL DEFERASIROX MONOTHERAPY FOR 1 YEAR IN 29 PATIENTS WITH TRANSFUSION-DEPENDENT ANEMIAS; A SUBGROUP ANALYSIS

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Background and Aims: Patients with transfusion-dependent anemias and iron overload who were entered into multicentre studies on deferasirox (Exjade) at University College London Hospitals (UCLH) had myocardial T2* performed at the Royal Brompton Hospital, as part of their routine monitoring. We have previously shown in 22 patients (16 β-thalassemia, 6 other chronic anemias) that treatment with deferasirox for 1 year at doses between 10 and 30 mg/kg/day is associated with an mean improvement in myocardial T2* of 6.4 ±1.76 ms, p=0.0026 (5.1ms geometric mean). (Porter et al., Blood 11, 3600, 2005) Patients and Methods: We now report a total of 29 patients who had myocardial T2* assessment before and after 1 year of treatment with deferasirox in studies 107 (randomised DFO vs deferasirox in β-thalassemia) and 108 (deferasirox monotherapy in β-thalassemia or other anemias). This is possible because an additional 7 patients, initially randomised to DFO on study 107, have now received deferasirox for 1 year. With larger anemias numbers, sub-analysis of trends in myocardial T2* response has been undertaken; in particular we have examined whether improvement in myocardial T2* is similar in patients with baseline myocardial T2* above or below the current reference normal range of 20ms. All means are reported as geometric means as the relationship between T2* and tissue iron has been considered to be logarithmic. Results: The geometric mean myocardial T2* in the 29 patients improved from 18.7ms (25%-75% CI, 11.7-29.3ms) at baseline to 23.0ms (CI, 13.5-37) (p=0.0005) after 1 year of treatment. If patients are divided into those with normal myocardial T2* values (T2*>20ms) and those with shortening of myocardial T2* (T2*<20ms) before deferasirox treatment, a significant improvement in both subgroups was observed. In the group of 15 patients who had normal myocardial values before treatment, the mean T2* improved from 30.3ms (CI, 25.5-36.3ms) to 36.9ms (CI, 31.5-44.8) within a year (p=0.006). In the group of 14 patients, who had abnormal myocardial T2* before treatment, a mean T2* improved from 11.2ms (CI, 9.6-12.4) to 13.9ms (CI, 10.11-16.3) (p=0.019) after one year of treatment with deferasirox. After 2 years of treatment with deferasirox only 15 patients are as yet available for baseline, 1-year and 2-year follow up analysis. In these patients, the mean myocardial T2* improved from 16.1ms (CI, 11.9-25.6) to 22.5ms (CI, 15.3-29.5) after 2 years treatment with deferasirox (p=0.005). Conclusion: Deferasirox is associated with an improvement in myocardial T2* after 1 year of treatment with deferasirox, in patients both with decreased baseline and normal baseline T2* values. Patients treated with deferasirox for 2 years maintained the improvement of the myocardial T2*. Prospective multicentre trials are now planned to study the effects of deferasirox on myocardial T2* further.
Non-Hodgkin's lymphoma
Chronic lymphocytic leukemia - Experimental

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NOTCH-MEDIATED ACTIVATION OF PI-3K IS NEEDED FOR LYMPHOMAGENESIS IN T CELL-SPECIFIC PTEN –/– MICE
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In the early stages of murine T cell development, cells go through two waves of proliferation: one mediated by IL-7 and stem cell factor, the other by triggering of the pre-TCR. An important downstream factor that is activated by IL-7 and the pre-TCR to positively regulate survival and proliferation is phosphatidylinositol-3-kine (PI-3K). Active PI-3K phosphorylates phosphatidylinositol-(4,5)-bisphosphate (PIP2) into phosphatidylinositol-(3,4,5)-triphosphate (PIP3), and this action is directly counteracted by Pten. Pten dephosphorylates PIP3 into PIP2. Previously we have shown that in mice before the age of 5 to 6 weeks, T cell-specific loss of Pten allows β- β lineage thymocytes to bypass IL-7 and pre-TCR mediated signaling, demonstrating a critical role of Pten in regulation of regional growth of developing thymocytes. After 5 to 6 weeks of age T cell-specific Pten– mice showed the first clinical signs of T cell lymphomagenesis; all mice died within 25 weeks. Incubation of freshly isolated thymocytes or of established thymocyte cell lines from these mice with PI-3K inhibitors wortmannin or LY294002 induced a block in proliferation, and induced apoptosis. These data indicated that loss of Pten alone is not sufficient to drive survival and proliferation; activated PI-3K is still needed. To investigate which factors are involved in PI-3K activation, we crossed T cell-specific Pten– mice with mice that lacked IL-7 (β common) and/or pre-TCR (CD3γ or RAG2) signaling. All resulting double and triple knockout mice developed lymphomas. Active PI-3K was still needed to ensure survival and growth, since thymocytes from these mice showed a block in proliferation and induction of apoptosis after incubation with wortmannin or LY294002. Similar results were obtained when cells were treated with the γ secretase inhibitor IX (DAPT), which specificallyblocks the Notch signaling pathway. Delta-like1-Notch signaling has been shown to phosphorylate Akt, a major player in cell survival. Therefore, we could be used as a model for the pathomechanism of 13q14.3 in CLL by the interaction of genetic lesions and epigenetic silencing.

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DYNAMIC MODIFICATIONS OF THE SURFACE B-CELL RECEPTOR LIGHT CHAIN IN CASES OF HAIRY CELL LEUKAEMIA OCCURRING AT EXTRAFOCAL Sites
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Background. Ig gene analysis delineates critical features of the clonal history of a B-cell tumor. After antigen interaction, mature B-cells undergo somatic mutation of the V-genes and isotype switch events, generally in the germinal center (GC). Receptor revision by secondary recombination of the V-genes with re-expression of rearranged Ig genes and isotype switch enzymes rarely occurs at this stage. From small series of cases, we have reported that most hairy cell leukemias (HCL) carry mutated VH-genes, with low levels of intrachromal heterogeneity, while a minor subset have unmutated VH-genes. Both subsets commonly have ongoing isotype switch events and express activation-induced cytidine-deaminase (AID). However they lack CD27 and CD88 CC markers, and CD25, essential for lymph node entry. Aims & methods. In an expanded series of HCL (60 cases) with VH-genes available, the expressed VL (32) tumor-derived genes were evaluated to probe more fully the differentiation status of the cell of origin. Results. The majority (35/44, 79.5%) co-expressed multiple Ig isotype proteins on the HCs. From fluorescence intensity analysis of VH, VHS family was most commonly expressed (60/60, 100%), with significant preference of the VH3-30 and VH3-33 members (p<0.005). Most HCL (46/60) carried variable tiers of mutations in the VH-genes (77.13-97.95% homology to germline), with low levels of intrachromal heterogeneity also documented in cases (15/60) with <2% deviation from germline. All 3/60 (5%) displayed complete unmutated VH-genes. Analysis of the light chains showed preferential use of surface κ chain (34/50, 62%), consistent with secondary rearrangement. VL-genes were evaluated in 16 κ and 16 λ expressing HCL. All (16/16) λ cases used J3 segment. Thirty of 32 cases carried mutated VL-genes (94,75%-99.6%) with low levels of intrachromal heterogeneity, while 2 cases carried completely unmutated VL-genes, reflecting heterogeneity in mutational status as for the VH-gene. Strikingly, cloning of the tumor VL revealed in-frame functional secondary rearrangements in 2/13 cases (Vκ1 & Vκ2 in Case R1, Vλm1d & Vλm2d in R2). Most likely occurring in different tumor cells. Primary and secondary rearrangements showed mutations (95.1 and 99.6% homology in R1; 97.6 and 99.6% homology in R2). In both cases, RAG1 re-induction was also identified by RT-PCR and sequence verification. Both cases expressed AID transcripts, displayed intrachromal mutational variation in the VH and/or the VL-genes and 1/2 cases had ongoing isotype switch events. These data suggest a dynamic, on-going modification of the B-cell receptor (BCR) in HCs, including receptor revision, which occurs in most cases likely in response to antigenic stimuli. N-glycosylation sites, commonly introduced by somatic mutation in the BCR of tumors of the GC, were not observed in the functional VH or VL-genes, to support the concept that tumor events occur outside the GC. Conclusions. These data confirm heterogeneity in the cell of origin in terms of mutational status, with a minor subset not affected. Restricted V-gene segment usage, and low levels of ongoing mutations with AID activated, coupled with the new observation of receptor revision and re-expression of RAG enzymes indicate that selective
bers of cytomegalovirus (CMV)-specific CD45RAvCD27 CD8+ cytotox-

have shown that in patients with B-CLL considerably expanded num-

ber of CD8+ CMV-specific cells results in a rather high responder rate when.

Aim: To test a novel bridging reagent to redirect CMV-specific CTL to.

specifically target B-CLL. This targeting construct is composed of a strep-

tavidin fused anti-CD20 single chain variable fragment (scFv) in combi-

nation with biotinylated MHC class I molecules containing CMV pp65.

peptide (HLA/CMV). Methods: We evaluated CD20-HLA/CMV induced.

lysis of B-CLL cells by CMV specific CTL. Results: Specific killing of.

CD8+ CTL with similar efficiency as B-CLL cells loaded with CMV-peptide.

was observed with CD20-HLA/CMV complexes. Furthermore, we used.


provides a new tool for tumor targeting of CMV-specific CTL in vitro.

B-CLL, its involvement in p53-independent apoptosis suggests this BH3-

protein may be a therapeutic target.

Aims. We studied the effects of seliciclib on apoptosis genes and Mcl-1.

protein interactions in B cell chronic lympho-

cytic leukemia (B-CLL), a malignancy with known aberrant apoptosis.


gene expression pattern (RT-MLPA) was investigated. Purified B-CLL.
cells (PBMC consisting of ≥90% B-CLL cells) and Ramos cells over-

expressing different apoptosis regulators were used in this study. Western.

blotting and immunoprecipitation assays were performed. Ramos.
cells were transduced with retroviral vectors expressing Noxa siRNA or

cell-only protein may be a therapeutic target.

Figure 1. Noxa RNAi afford selective resistance to seliciclib.

THE CYCLIN-DEPENDENT KINASE INHIBITOR SELICICLIB ENGAGES THE

MITOCHONDRIAL APOPTOSIS PATHWAY VIA THE MCL-1/NOXA AXIS IN B-CLL

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Backgrounds. Seliciclib (R-roscovitine) is a cyclin-dependent kinase

inhibitor in clinical development. It triggers apoptosis by inhibiting de

novo transcription of the short-lived Mcl-1 protein. It is not known how

Mcl-1 degradation triggers Bax or Bak activation that is required for

most forms of cell death. Aims: We studied the effects of seliciclib on

apoptosis genes and Mcl-1 protein interactions in B cell chronic lympho-

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Figure 1. Noxa RNAi afford selective resistance to seliciclib.
Pharmacogenetics and molecular targeting

1005
PHARMACOGENETIC ANALYSIS OF POLYMORPHISMS IN CYP3A4, CYP3A5, GSTP1, GSTM1, GSTT1 AND MDR1 GENES FOR SURVIVAL AND THERAPY RELATED TOXICITY IN MULTIPLE MYELOMA

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Erasusmc, ROTTERDAM, Netherlands; ErasmusMC-Daniel den Hoed Cancer Center, ROTTERDAM, Netherlands; Radboud University Medical Center, Nijmegen, NIJMEGEN, Netherlands; University Medical Center Utrecht, UTRECHT, Netherlands; University Medical Center Groningen, Groningen, GEN- GEN, Netherlands

Background. Cytochromes P450 (CYP450) and glutathione-S-transferases (GSTs) are drug metabolizing enzymes involved in detoxification of chemotherapeutic agents. Genetic polymorphisms in these genes are frequent and may alter the metabolism of anti-cancer drugs. Among these, the CYP3A4*1B (290A>G) polymorphism affects the promoter region. Polymorphisms in the CYP3A5 (6986A>T) the 2435C>T polymorphism in the MDR1 gene alters protein expression. Homozygous deletions in the GSTM1 and GSTT1 genes result in absence of the enzyme. We hypothesized that inherited mutations in these enzymes may result in different treatment response and toxicity in multiple myeloma. Aims. We investigated the relevance of different genetic polymorphisms of these enzymes for clinical outcome in multiple myeloma patients treated in the HOVON 24 prospective clinical phase III study, a clinical trial comparing single with double intensive therapy. Methods. Genetic polymorphisms were determined in DNA isolated from peripheral blood of 211 myeloma patients treated in the HOVON 24. Polymorphisms in different genes were detected using a restriction fragment length polymorphism (RFLP)-PCR and frequencies were analyzed with outcome. Results. For the CYP3A4, GSTT1, GSTM1 and MDR1 genes no significant influence was observed for partial remission (PR), complete remission (CR), event free survival (EFS), progression-free survival (PFS), time to progression (TTP), overall survival (OS) and toxicity. However, patients with two variant alleles for the GSTP1 gene had significantly lower remission rates (p=0.01). Patients with the homozygous genotype of the CYP3A5 mutant allele showed improved PFS (p=0.04) and OS (p=0.01) and an increase of TTP (p=0.03). Summary/ conclusions. This is the first study that shows a significant association between the CYP3A5/3 polymorphism and outcome for a cohort of MM patients treated in a clinical trial comparing single with double intensive therapy. Patients with two variant alleles for the GSTP1 gene had significantly lower remission rates, although this did not translate into significant differences in EFS, PFS, TTP and OS. Currently the results of this biological study are evaluated using a multivariate comparison of these data with the clinical prognostic factors. Secondly, these results will be validated in other treatment settings in subsequent HOVON 50 trial. The results will be presented in a comprehensive analysis of both clinical and pharmacogenetic variables.

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EXPOSURE OF LEUKEMIC CELLS TO ANTHRACYCLINES INDUCES RAPID AND BROAD UPGREGULATION OF ATP BINDING CASSETTE TRANSPORTERS

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Drug efflux by ATP-binding cassette (ABC) transporters is a well-established mechanism by which leukemic cells evade chemotherapeutic eradication. The role of most ABC transporters in chemoresistance of leukemic cells, however, remains unknown. The dynamics of ABC transporters after short-term drug exposure with anthracyclins (mitoxantrone and daunorubicin) in leukemic progenitor cells (KG1a cell line and primary AML CD34+ cells) by real-time RT-PCR on micro fluidic cards. In KG1a cells significant induction (>2-fold) of many ABC transporters was observed within 72 hours of exposure to both mitoxantrone and daunorubicin (25 and 33 transporters respectively). Of these induced transporters, 12 transporters show an upregulation of more than 20-fold up to 850-fold for ABCA4 after exposure to mitoxantrone, and after exposure to daunorubicin the induction increases up to 2800-fold for ABCA6. Among the top 12 of highest induced genes, 8 transporters were overlapping between mitoxantrone and daunorubicin treated cells, including the known drug resistance genes MDR1 (ABCB1) and ABCB11. The remaining highest up regulated genes are currently not associated with drug resistance. Rapid and broad induction of ABC transporters upon exposure to anthracyclins was confirmed in primary CD34+ leukemic cells in vitro (n=2 patients). In the first patient sample 24 and 15 ABC transporters were more than two-fold up regulated after mitoxantrone and daunorubicin exposure respectively. In the second patient sample 11 and 15 ABC transporters were up regulated after exposure to mitoxantrone and daunorubicin respectively. There was a large overlap between the induced transporters after exposure to mitoxantrone and daunorubicin between patient samples and within patient samples. In the top ten of most induced genes were 7 transporters known to be involved in drug resistance, including the above mentioned 3 drug transporters induced in the KG1a cell line. Also in the patient samples the remaining transporters are currently not associated with drug resistance. These data show that short-term drug exposure rapidly induces a large range of ABC transporters in leukemic progenitor cells. These undisclosed known drug transporters may be previously not associated with drug resistance. The findings challenge the rationale of inhibition of single transporters to circumvent drug resistance of leukemic progenitors and warrant further research into the role of novel ABC transporters in chemoresistance of leukemic cells.

1007
TARGETED INACTIVATION OF GERANYLGERANYLTRANSFERASE TYPE I RESCUES MICE FROM LETHALITY INDUCED BY ONCOCENIC K-RAS EXPRESSION IN GRANULOCYTES AND MONOCYTES

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Backgrounds. Ras proteins are isoprenylated at a carboxyl-terminal CAAX motif by farnesyltransferase (FTase) and geranylgeranyltransferase (GGTase) enzymes. GGTase transfers a geranylgeranyl moiety to Ras proteins once its CAAX motif is cleaved. Ras proteins are often mutated, resulting in constitutive activation, rapid and broad induction of ABC transporters, 12 transporters show an upregulation of more than 20-fold. In the absence of Ras proteins, cells develop leukocytosis, splenomegaly, infiltration of cells in the liver, fibroblasts to enter cell cycle arrest. Next, we developed a new mouse model of leukemia, based on K-Ras-induced leukemia. Methods. We have generated mice with a Cre-inducible GGTase-I knockout allele (Pgg1bflx). Moreover, we have developed a new mouse model of leukemia, based on Cre-loxP techniques, where the expression of oncogenic K-Ras was targeted to granulocytes and monocytes. For this, we used mice with an oncogenic mutation (G12D) in the Kras2 locus (Kras2LSL). In the absence of Kras2LSL, the spleen was significantly reduced. Inactivation of Gtase-I would block the development of K-Ras-induced leukemia. Methods. We have generated mice with a Cre-inducible GGTase-I knockout allele (Pgg1bflx). Moreover, we have developed a new mouse model of leukemia, based on Cre-loxP techniques, where the expression of oncogenic K-Ras was targeted to granulocytes and monocytes. For this, we used mice with an oncogenic mutation (G12D) in the Kras2 locus (Kras2LSL). In the absence of Cre, this Kras2LSL mice are lethal. Induction of Cre turns on the expression of Kras2LSL. We have bred mice with the Kras2LSL allele and a Cre transgene driven by the lysozyme M promoter (Lysm-Cre). In these mice, Cre expression, and subsequently Kras2LSL, expression, is targeted to granulocytes and monocytes. Finally, to determine if inhibition of GGTase-I would block the development of K-Ras-induced leukemia, we have used Cre to simultaneously activate the expression of Kras2LSL and inactivate the expression of Pgg1b in the same cells. In this way, we determined if the absence of Pgg1b would prevent the development of K-Ras-induced leukemia. Results. We found that the Cre-induced knock-out of Pgg1b abolished GGTase-I activity and was compatible with cell viability in both fibroblasts and myeloid cells and caused proliferating fibroblasts to enter cell cycle arrest. Next, we developed a new mouse model of leukemia, where Kras2LSL expression was targeted to granulocytes and monocytes. We found that the Kras2LSL-Lysm-Cre mice developed leukocytosis, splenomegaly, infiltration of cells in the liver,
FLT3 MUTATED PEDIATRIC ACUTE MYELOID LEUKEMIA (AML) SAMPLES ARE SENSITIVE TO THE TYROSINE KINASE INHIBITOR SU11657

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Despite intensive treatment regimens only 60% of pediatric AML patients survive. Therefore novel treatment strategies to improve the outcome of pediatric AML are required. Almost 20% of pediatric AML patients harbor a FLT3 mutation (12% FLT3/ITD and 7% FLT3 D835 point mutations). Patients with a FLT3/ITD mutation have a poor prognosis. Tyrosine kinase inhibitors are novel drugs specifically targeting activated tyrosine kinases. SU11657 is one of these novel drugs and is a selective inhibitor of the tyrosine kinase receptors FLT3, KIT, PDGF and VEGF-R2. SU11657 is comparable to the currently approved SU11248 (sunitinib malate, Sutent®). In a phase I trial of sunitinib malate in AML all patients with FLT3 mutations (n = 4) had complete clinical and partial morphologic responses compared with 2 of 10 evaluable patients with wild-type FLT3. These responses were of short duration, although longer in patients with mutated than wild type FLT3. In this study we investigated whether primary pediatric AML samples were sensitive to SU11657 in vitro and whether the effects of SU11657 are related to mutations in FLT3 and KIT. We studied 70 pediatric AML samples for FLT3/ITD mutations using genescan analysis and FLT3 D835 using light-cycler analysis. The KIT mutational analysis is in progress. These 70 pediatric AML samples were also tested for in vitro sensitivity to SU11657 using the 4 day total cell kill MTT assay (concentration range 0.0098 - 10 µM). Two measures of sensitivity were calculated: 1. The LC50 value (the concentration at which 50% of the cells is killed); 2. The percentage of cells surviving at 0.625 µM SU11657 (the concentration which discriminates best between sensitive and resistant samples). A FLT3/ITD mutation was detected in 20 samples (29%) and a FLT3 D835 mutation in 4 samples (6%). There was a 1000 fold difference in LC50 value between the most sensitive and the most resistant sample. Dose-response curves differed from what is normally observed in the MTT assay, as even high concentrations did not result in impressive cell-kill, as was also observed by others for FLT3 inhibitors when testing primary samples. AML samples without FLT3 mutations were resistant to SU11657 (median LC50=7.4 µM, median cell survival at 0.625 µM SU11657=91%). In contrast FLT3/ITD positive samples were significantly more sensitive to SU11657 (median LC50=9.9 µM (p=0.057), median cell survival at 0.625 µM SU11657=66% (p<0.0001)). The 4 FLT3 D835 mutated samples were sensitive to SU11657 (median LC50=2.4 µM (FLT3 negative vs. D835 p=0.16), median cell survival at 0.625 µM SU11657=64% (FLT3 negative vs. FLT3 D835 p=0.017)). Despite these differences there was overlap in individual LC50 values, with some FLT3 non-mutated samples being relatively sensitive, and some FLT3 mutated samples being relatively resistant to SU11657. We are currently performing KIT mutational analysis which may explain this sensitivity in non-FLT3 mutated samples. In conclusion, there is large interpatient variation in sensitivity to SU11657. Both FLT3/ITD and FLT3 D835 positive pediatric AML samples were more sensitive to SU11657 in vitro than FLT3 negative samples.

DEVELOPMENT OF AN EFFECTIVE SAFETY SWITCH FOR SELECTIVE ELIMINATION OF HUMAN T CELLS IN VIVO AFTER ADOPTIVE TRANSFER

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Backgrounds. Adoptive transfer of T cells is frequently associated with unwanted side effects. In order to make these effects less toxic we introduce a safety switch into the cells that permits their selective in vivo elimination. The human CD20 gene in combination with CD20 antibodies was recently proposed as a novel safety switch. In such a system, T cells may be genetically modified with a CD20-encoding vector prior to adoptive transfer. If necessary, CD20-transgenic cells can be eliminated in vivo through administration of CD20 antibodies, such as the chimeric antibody rituximab (RTX) that is currently used to treat CD20 lymphoma cells. RTX activates the complement system and recruits immune effector cells, resulting in rapid death of CD20+ cells. Recently, a novel human CD20 antibody, Humab 7D8, was shown to have superior activity over RTX. Aims and Methods. In this study a set of CD20-encoding retroviral vectors was generated, which either lacked or contained one or both of two regulatory elements: 1) the woodchuck posttranscriptional regulatory element (WPRE) to increase CD20 expression, and 2) the chicken hypersensitivity site 4 insulator element (INS) to achieve a position independent expression of CD20 and to increase the safety profile of the vector by preventing activation of cellular (onco)genes by the retroviral enhancer. Results. We found that the level of CD20 expression obtained with vectors containing INS was 2-fold lower than with vectors lacking INS. Additional inclusion of WPRE restored the level to that of the vector without INS. In addition, INS greatly enhanced the homogeneity of CD20 expression in T cells. Moreover, after 3 months in culture, all cells generated with CD20-INS had retained CD20 expression, while 60% of cells transduced with the control CD20 vector had lost CD20 expression. Complement dependent cell kill (CDC) of both RTX and Humab 7D8 was dependent on the level of CD20 expression (p<0.01). However, while very low CD20-expressing cells were completely resistant against RTX they could be effectively killed by Humab 7D8. For maximal killing of CD20-high cells a 100-fold lower dose of Humab 7D8 was required, compared to RTX. In vivo efficacy was studied through bioluminescent imaging of luciferase+ CD20-transgenic T cells. After transfer of CD20+ cells in immune deficient Rag2-/-γc-/- mice, both CD20 antibodies were capable of eliminating >99% of CD20+ cells, prolonging survival of mice from 20 till 42 days. Summary. We developed a safe vector that leads to homogeneous CD20 expression in human T cells. These cells can be killed effectively in vivo with Humab 7D8, a recently developed CD20 antibody. This system will be applicable to other approaches that require inclusion of a safety switch in ex vivo modified cells.
Cancer genetics and cytogenetics in lymphoid diseases

1010
MOLECULAR KARYOTYPING BY HIGH RESOLUTION ARRAY CGH UNCOVERS AMPLIFICATIONS AND HOMOZYGOUS DELETIONS IN CD138 + SELECTED PRIMARY MULTIPLE MYELOMA CASES

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Multiple Myeloma (MM) is a malignancy of clonal plasma cells with a wide variability in clinical features, responses to treatment, and survival times among patients. In 50% of the cases, the neoplastic transformation begins with a chromosomal translocation that juxtaposes the IGH gene locus to an oncogene. In addition, other genetic aberrations, as gains and/or losses of genomic regions (including trisomies and monosomies) are frequent but less characterized and they may contribute to the tumour phenotype. Our objective was to characterize copy number changes present on CD138 + multiple myeloma primary samples by means of DNA hybridization onto high resolution array CGH platforms. We conducted a high resolution analysis of copy number changes on MAC'S sorted CD138 + myeloma cells. Twenty-six newly diagnosed MM samples at diagnosis were included in the study. 85% of the patients carried a normal karyotype at diagnosis. The median age of our patients was 67.5 years (range: 34-76). There were 16 men and 10 women in our series. The presence of IGH rearrangement has been analyzed using LSI IGH Dual Colour, Break Apart Probe (Vysis). For molecular karyotyping Human Genome CGH Microarray 44A/B platform from Agilent Technologies (Palo Alto, CA, USA) was used. This platform contains 44,000 60-mer oligonucleotides covering the human genome with an average resolution of 45 Kb. CGH-Analytics 3.2.25 Agilent Technologies (Palo Alto, CA, USA) was used for the array analysis. RESULTS. Genomic copy number analysis, performed on selected cells, allowed the identification of a high number of deletions and gains. FISH screening revealed that 9 out 26 (35%) samples harboured an IGH rearrangement. We have discovered 267 copy number changes with a median of 8.5 changes per case, ranging from 2 to 26 copy number changes per case. By this CGH approach, we characterized whole chromosome 3, 5, 7, 9 and 15 gains in 50% of the samples. This defined the hyperdiploid group in our series. Chromosome 18 deletions have been found in 58% of the cases. Gains of chromosome 19p, 1q gain, and a novel duplication of Xq21-qter were found to be among the most frequent aberrations. In addition to big structural changes, we have also identified small rearranged regions (below 500 Kb of size). Of great genetic relevance was the finding of homozgyous deletions in chromosomes 6q, 11q, 13q and Xq, and the description of genomic amplifications in 6q, 9q, 16q and 17q. Finally we have established 68 common minimal rearranged regions that will be used in unsupervised and supervised clustering. This is the first time that high resolution array CGH analysis is carried out on CD 138+ on MM primary samples. This approach has allowed us to identify copy number changes in 100% of the samples and has made possible the identification of great genetic relevance homozgyous deletions and amplifications in MM.

1011
MOLECULAR ANALYSIS OF PATIENTS WITH T-ALL USING ARRAY-CGH ENRICHED IN PROBES COVERING TYROSINE KINASE GENES

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In T-ALL a wide variability in clinical features, response to treatment and survival times among patients. In 50% of the cases, the neoplastic transformation begins with a chromosomal translocation that juxtaposes the IGH gene locus to an oncogene. In addition, other genetic aberrations, as gains and/or losses of genomic regions (including trisomies and monosomies) are frequent but less characterized and they may contribute to the tumour phenotype. Our objective was to characterize copy number changes present on CD138 + multiple myeloma primary samples by means of DNA hybridization onto high resolution array CGH platforms. We conducted a high resolution analysis of copy number changes on MAC’S sorted CD138 + myeloma cells. Twenty-six newly diagnosed MM samples at diagnosis were included in the study. 85% of the patients carried a normal karyotype at diagnosis. The median age of our patients was 67.5 years (range: 34-76). There were 16 men and 10 women in our series. The presence of IGH rearrangement has been analyzed using LSI IGH Dual Colour, Break Apart Probe (Vysis). For molecular karyotyping Human Genome CGH Microarray 44A/B platform from Agilent Technologies (Palo Alto, CA, USA) was used. This platform contains 44,000 60-mer oligonucleotides covering the human genome with an average resolution of 45 Kb. CGH-Analytics 3.2.25 Agilent Technologies (Palo Alto, CA, USA) was used for the array analysis. RESULTS. Genomic copy number analysis, performed on selected cells, allowed the identification of a high number of deletions and gains. FISH screening revealed that 9 out 26 (35%) samples harboured an IGH rearrangement. We have discovered 267 copy number changes with a median of 8.5 changes per case, ranging from 2 to 26 copy number changes per case. By this CGH approach, we characterized whole chromosome 3, 5, 7, 9 and 15 gains in 50% of the samples. This defined the hyperdiploid group in our series. Chromosome 18 deletions have been found in 58% of the cases. Gains of chromosome 19p, 1q gain, and a novel duplication of Xq21-qter were found to be among the most frequent aberrations. In addition to big structural changes, we have also identified small rearranged regions (below 500 Kb of size). Of great genetic relevance was the finding of homozgyous deletions in chromosomes 6q, 11q, 13q and Xq, and the description of genomic amplifications in 6q, 9q, 16q and 17q. Finally we have established 68 common minimal rearranged regions that will be used in unsupervised and supervised clustering. This is the first time that high resolution array CGH analysis is carried out on CD 138+ on MM primary samples. This approach has allowed us to identify copy number changes in 100% of the samples and has made possible the identification of great genetic relevance homozgyous deletions and amplifications in MM.

The response to cytotoxic drugs varies amongst different subtypes of childhood acute lymphoblastic leukemia (ALL). We studied the expression of 70 apoptosis genes by Affymetrix U133A GeneChip microarrays in leukemic cells taken at initial diagnosis of 190 children with ALL. The expression of 44 out of these 70 genes differed significantly between T-lineage and B-lineage ALL. 22 genes differed in hyperdiploid versus non-hyperdiploid, 16 in TEL-AML1 positive versus negative, and 18 in E2A-rearranged versus negative B-lineage ALL. The data indicate that the expression of apoptosis genes highly differs between these leukemic subtypes. Expression of MCL1 and DAPK1 was significantly associated with prednisolone resistance, whereas BCL2L13, HRK and TNF were related to L-asparaginase resistance. Multivariate analysis including known risk factors revealed that BCL2L13 expression was an independent prognostic factor (p=0.011). The same trend was observed in a validation group of 92 children with ALL treated on a different protocol at the St. Jude Children’s Research Hospital (p=0.051). In conclusion, apoptosis genes are differentially expressed between subtypes of acute lymphoblastic leukemia. Out of 70 studied apoptosis genes, only 5 genes were associated with cellular drug resistance in childhood ALL. Functional studies addressing the causal relationship between these genes and drug resistance are currently being performed.

Background. Molecular analysis of T-cell acute lymphoblastic leukemia (T-ALL) has provided evidence for a stepwise alteration of tyrosymes during transformation to leukemic T-cells. Genetic alterations in hematopoietic precursor cells lead to loss of cell cycle control, impaired differentiation, proliferation and survival advantages, and unlimited self-renewal capacity. These defects include inactivation of CDKN2A (P16) present in 96% of the patients, deregulated expression of transcription factors, and mutation of NOTCH1 present in 56% of patients. The molecular lesions leading to the proliferative and survival advantages of T-ALL cells are less well characterized and remain unknown in 80% of the T-ALLs. We have previously shown that cryptic deletions and amplifications can result in the generation of fusion genes. Examples of these are the cryptic 800 kb deletion on chromosome 4q12, and amplification of a 500 kb region of 9q34, resulting in the generation of FIP1L1-PDGFRα and NUP214-ABL1 fusion genes. Aim. Our aim was to set up a genome-wide analysis of T-ALL in order to detect cryptic deletions and amplifications, with a special focus on the 90 protein tyrosine kinase genes present in the human genome. Methods. We used the array-CGH (micro-array comparative genomic hybridization) technology with slides containing genomic BAC probes spaced every 1 Mb over the human genome. An additional 480 probes were added covering the genomic locations of each of the 90 protein tyrosine kinase genes. Results. We performed array-CGH on 20 T-ALL cases. An interstitial deletion on chromosome 9p24 directly upstream of JAK2 was identified in 1 case. The deletion was confirmed by FISH. Quantitative PCR analyses indicated that the deletion was 700 kb in size including exons 1-3 of JAK2. Molecular analyses to characterize the possible presence of a fusion transcript involving JAK2 are in progress. No other rearrangements involving tyrosine kinase genes were observed in 19 other T-ALL cases, suggesting that cryptic deletions or amplifications involving tyrosine kinase genes are relatively rare in T-ALL. The most frequent aberration was the deletion of CDKN2A (14 cases). MYB duplication was found in two cases, and was confirmed by quantitative PCR. PTEN deletion was present in one case. Other unbalanced aberrations of various size were detected: del(6q) in 6/17 cases ranging from 5 to 35 Mb, del(9p) in 4/17 cases ranging from 4 to 43 Mb, dup(7q) in 2/17 cases and, dup(12q), del(7p), dup(9p) and del(12p) in one case each. Some of these rearrangements were not observed by standard cytogenetics. Conclusions. We detected a novel cryptic rearrangement of JAK2 in one T-ALL case, and duplication of MYB in two T-ALL cases. Molecular analysis of these cases, and array-CGH analysis of an additional 20 T-ALL cases and 10 T-ALL cell lines is ongoing.
D-type cyclins are key regulators of progression through G1 phase of the cell cycle. Strong expression of at least one of the three D cyclins is common in human cancers. However, while the cyclin D1 and D3 genes (CCND1 and CCND3) are recurrently involved in genomic rearrangements, especially in mantle-cell lymphoma and multiple myeloma, no clear involvement of the cyclin D2 gene (CCND2) has been reported to date in human malignancies. In T-cell acute lymphoblastic leukemia (T-ALL), the T-cell receptor genes TCRA/D and TCRA/B are frequently involved in chromosomal rearrangements and deregulate oncogenes. In order to identify new chromosomal rearrangements and oncogenes in human T-ALL, we performed an interphasic FISH screening of T-ALL cases using TCR flanking probes. By this approach, we identified two new chromosomal translocations: t(7;12)(q34;p13) and t(12;14)(p13;q11), involving the TCRA/D and TCRA/B loci, respectively. Molecular analysis of the breakpoint derivative sequences demonstrated the involvement of the CCND2 locus at 12p13. Expression analysis using RT-qPCR and immunoblotting demonstrated dramatic cyclin D2 overexpression in the translocated cases (n=3) compared to other T-ALLs (total, n=86), whereas other genes located near the translocation breakpoints were not deregulated on microarray analysis. To further evaluate expression in T-ALL with respect to normal T-cell differentiation, we analyzed CCND2 expression in purified subpopulations from normal human thymus. CCND2 levels were downregulated through progression from the early stages of normal human T-cell differentiation and transition to β-selection. In the most immature T-ALLs, a moderate CCND2 expression was observed, consistent with their differentiation stage, while low CCND2 levels were observed in T-ALL. By contrast, the massive and sustained expression in the CCND2-rearranged T-ALL cases strongly suggested oncogenic function due to the TCR translocation. T-ALL oncogenesis is a multi-step process. We here found that the TCR-CCND2 translocations were associated with other oncogene expression (TAL1, HOXA, or TLX3/HOX11L2), NOTCH1 activating mutations, and/or CDKN2A/p16/ARF deletion, showing that cyclin D2 dysregulation could contribute to multi-event oncogenesis in various T-ALL groups. In conclusion, this report is the first clear evidence of a direct involvement of cyclin D2 in human cancer due to recurrent somatic genetic alterations. This reinforces the view that the strong expression of cyclin D2 which is detected in various types of cancer including T-ALL cases can contribute to oncogenesis, and points to cyclin D2 as a potential target for therapy in these tumors.

Mantle cell lymphoma (MCL) is a B-cell neoplasm associated with the translocation t(11;14)(q13;q32) resulting in an overexpression of cyclin D1. In addition, high numbers of secondary genomic aberrations were shown by chromosomal banding analysis, comparative genomic hybridization (CGH) and array-CGH studies. The aim of the present study was a precise delineation of chromosomal consensus regions for the most frequent genomic aberrations in MCL in order to provide a basis for the identification of candidate genes. For this purpose, a dedicated ‘MCL-array’ consisting of 4126 DNA-probes was developed. 21 genomic regions, known to be recurrently affected by genomic aberrations in MCL were covered by high resolution physical maps with a total of 3359 DNA-probes. These regions encompass: 1p13-1p22, 4q24-3q29, 6p22-6p25, 6q23-6q27, 7p15-7p22, 8p21, 8q24, 9p21-9p22, 9q21-9q22, 10p11-10p15, 11q13, 11q22-11q23, 12p12-12p13, 12q12-12q21, 13q14, 15q33-15q34, 14q32, 15q25-15q26, 17p11-17p15, 18q21-18q23, 22q11-22q13. Additionally 767 probes linearly covering the genome in a distance of 4 megabasespairs were used for the normalization of the data. A first series of cryopreserved tumor samples in 25 patients with t(11;14)-positive MCLs were analyzed. In all cases, genomic aberrations were identified. The most frequent genomic gains mapped to chromosome arm 3q (14 cases) followed by gains of 7p, 11q and 18q (7 cases each). The most frequent genomic deletion affected chromosome arm 18q (18 cases). Further deletions mapped to chromosome arms 11q (12 cases), 1p and 9p (11 cases each). The smallest consensus region for genomic gains was defined for 10p13 with a size if 600 kilobasepairs, containing the BMI1 gene. The consensus region on 8q24 was narrowed down to a size of 1.0 megabasepairs, containing the MYC gene. The smallest minimal deleted regions with a size of 600 kilobasepairs each mapped to 8p21 and 9p21, containing TNFRSF10B and CDKN2A/CDKN2B. The consensus region on 12p13 was narrowed down to a size of 1.1 megabasepairs, containing CDKN1B. These data demonstrate the usefulness of a custom made high resolution microarray as a precise tool for the delineation of genomic consensus regions in MCL. Completing a larger series of MCL these data will provide a more detailed basis for the identification of altered chromatin segments, which can contribute to the identification of candidate genes in this tumorenosity.
Allogeneic stem cell transplantation - Clinical

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LOW TREATMENT-RELATED MORTALITY AND RAPID REGRESSION OF BONE MARROW FIBROSIS AFTER REDUCED INTENSITY ALLOGENEIC STEM CELL TRANSPLANTATION IN PATIENTS WITH MYELOFIBROSIS, AN INTERIM ANALYSIS OF A PROSPECTIVE STUDY OF THE CHRONIC LEUKAEMIA WORKING PARTY OF THE EUROPEAN GROUP OF BLOOD AND MARROW TRANSPLANT

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Background. Allogeneic stem cell transplantation is the only curative approach for patients with myelofibrosis. Due to the high treatment related mortality only younger patients are candidate for this treatment approach. Aim. To investigate in a prospective, multicenter study the effect of a reduced-intensity conditioning regimen with busulfan (10 mg/kg), fludarabine (180 mg/m^2) and anti-thymocyte globulin, followed by allogeneic stem cell transplantation in patients with myelofibrosis. Methods. At time of analysis (3/2006) 37 patients with related (n=16) or unrelated donors (n=21) were evaluable for toxicity, treatment related mortality and efficacy According to the Lille score, myelofibrosis was classified as low (n=7), intermediate (n=22) or high risk (n=8). The median age of the patients was 53 years (range, 32-67). Stem cell source was peripheral blood stem cells (n=36) or bone marrow (n=1) from HLA-matched donor (n=32) or HLA-mismatched donor (n=5). Results. No primary graft failure was observed. The median time until leukocyte (>1.0 x10^9/L) and platelet (> 20 x10^9/L) engraftment was 17 days and 22 days, respectively. The leukocyte engraftment was faster in splenectomized patients (p=0.001). Acute graft-versus-host disease (GVHD) grade II and IV occurred in 21% and 8% of the cases, and 21% of the patients experienced chronic GVHD. The treatment-related mortality at one year was 6%. The cumulative incidence of relapse at three years was 18%. After a median follow-up of 12 months, the estimated three-year overall and disease-free survival was 85% and 75%, respectively. The three-year estimated disease-free and overall survival was 77% and 85%, respectively. The disease-free survival was higher in low risk than in intermediate/high risk patients (p<0.05). In 20 patients, the dynamics of bone marrow changes could be monitored one month, six months and one year after stem cell transplantation by sequential bone marrow trephine biopsies. A total regression of the pre-transplant fibrosis was completed in the post-transplant period after about six months while the extent of osteosclerosis did not change significantly during observation time. The CD34 progenitor cells in bone marrow (n=1) and peripheral blood (n=15) at the day of transplantation increased rapidly to normal values in all responding patients. Conclusions. Reduced-intensity conditioning in patients with myelofibrosis provide rapid and sustained engraftment with a low one-year treatment-related mortality resulting in an encouraging three-year overall and disease-free survival. Allogeneic stem cell transplantation results in rapid regression of fibrosis and in rapid decline of CD34 progenitor cells in the bone marrow.

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HAPLOIDENTICAL HEMATOPOIETIC STEM CELL TRANSPLANTATION WITHOUT IN VITRO T CELL DEPLETION FOR THE TREATMENT OF HEMATOLOGICAL MALIGNANCIES

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Backgrounds. Many patients who require allogeneic hematopoietic stem cell transplantation (allo-HSCT) do not have a human leukocyte antigen (HLA)-matched donor. HSCT from HLA-mismatched family donors is associated with lower long-term survival and delayed immune reconstitution, especially if the T cells are depleted. Aims. Here we describe a method for haploidentical allo-HSCT from family members without in vitro TCD, designed to overcome the HLA barrier and reduce transplant-related complications. Results. In the present study, the method for haploidentical allo-HSCT without in vitro TCD involves sequential, in vivo modulation of T cell functions in the recipient and the donor, and adjustment of the dose of donor HSCT. This protocol has three elements: anti-human thymocyte immunoglobulin (ATG) for the prevention of GVHD, a combination of G-PCBs and G-BM, and donor treatment with recombinant human G-CSF. There are 176 patients, including 88 with high-risk malignancies, underwent transplantation from an HLA-haploidentical family donor with 1-3 mismatched loci. All patients achieved sustained, full donor chimerism. The cumulative incidence for to acute GVHD was 22.7%, which was not associated with HLA disparity. The cumulative incidence for extensive cGVHD was 46.9%. The 2-year probability of relapse was 12.2% in the standard risk group and 38.9% in high-risk group. The probability of 1-year and 2-year leukemia-free survival (LFS) was in 71% and 68.2% for standard-risk patients and 54.3% and 42.1% for high-risk patients (p=0.0009) respectively. Summary/Conclusion: These results show that G-BM combined with G-PCBs from haploidentical family donors, without in vitro TCD, could be used as a good source of stem cells for allogenic HSCT. The new HSCT regimen described here allows use of a haploidentical family members as donors, a strategy likely to be much more important in the future, for the increasing numbers of Chinese patients, and those of other ethnicities, who are the only child in the family.

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PURIFIED T DEPLETED PERIPHERAL BLOOD AND BONE MARROW CD34+ TRANSPLANTATION FROM HAPLOIDENTICAL MOTHER TO CHILD WITH THALASSEMIA

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Approximately 60% of thalassemic patients can not apply to 'gene therapy today' which the insertion of one allogenic HLA identical stem cell into the empty bone marrow as the vector of the normal gene for β-globin chain synthesis. We present the results of a prospective approach. Here we describe a haploidentical mother as the donor of hematopoietic stem cells assuming that the immunotolerance established during the pregnancy will help to bypass the HLA disparity and allow the hematopoietic allogeneic reconstitution in the thalassemic recipient of the transplant. We have employed a new preparative regimen for the transplant in fourteen thalassemic children aged 3 to 12 years (median age 5 years) using T cell depleted peripheral blood stem cell (PBSCTs) plus bone marrow (BM) stem cells. All patients received hydroxyurea (OHU) 60 mg/kg and azathioprine 3 mg/kg from day -59 until day-11, fludarabine (FLU) 30 mg/m^2 from day -17 to day -11, and cyclophosphamide (CY) 200mg/kg, Thiopeta 10 mg/kg and ATG S Organ 2.5 mg/kg, followed by a CD34 + cell depleted (Clinimacs system) granulocyte colony stimulating factor (G-CSF) mobilized PBSCT from their HLA haploidentical mother. The purity of CD34+ cells after MACS sort six months and one year after stem cell transplantation by sequential bone marrow trephine biopsies. A total regression of the pre-transplant fibrosis was completed in the post-transplant period after about six months while the extent of osteosclerosis did not change significantly during observation time. The CD34 progenitor cells in bone marrow (n=1) and peripheral blood (n=15) at the day of transplantation increased rapidly to normal values in all responding patients. Conclusions. Reduced-intensity conditioning in patients with myelofibrosis provide rapid and sustained engraftment with a low one-year treatment-related mortality resulting in an encouraging three-year overall and disease-free survival. Allogeneic stem cell transplantation results in rapid regression of fibrosis and in rapid decline of CD34 progenitor cells in the bone marrow.
results regarding GvHD. Although there is emerging evidence that Tregs are associated with a poor outcome in cancer patients, none of these studies has investigated the role of Tregs in leukemia relapse post-SCT. Aims. To quantify CD4CD25High regulatory T-cells in post-SCT patients and correlate their levels with clinical outcome. Materials and Methods. We performed a cross-sectional study at a single institution. We enumerated and characterized peripheral blood CD4CD25High Tregs in 76 patients post-SCT for CML by IACS analysis. A subset of 44 patients were then selected to assess the outcome of patients in whom imatinib was discontinued after 12.2 (range: 10.1-16.5) months. Estimated probability of remission (PCR negativity) was determined in each patient. Results. Patients post-SCT had higher levels of Treg than normal donors (median 1.5% vs. 0.87%, p<0.01) and untreated CML (median 1.5% vs. 2.7%, p<0.0001). In the multiple regression analysis only the time post SCT (before or after 18 months) and disease status (molecular remission versus relapse) were predictive for increased Tregs (Coef -2.994, p=0.004 and Coef -2.935, p=0.020 respectively). No association with Treg levels and GvHD was found. The logistic regression analysis performed in 48 patients that had not received DLI post SCT confirmed that increased Tregs, both as percentage or absolute numbers, were the only predictive variable for relapse (exp 1.44, p=0.011). Conclusions. A substantial expansion of Tregs occurs early after allogeneic SCT and the presence of high numbers of Tregs 18 months after transplant is predictive of leukemic relapse. Although the increment might initially have an advantageous effect on graft rejection, our data suggest that Tregs exert an inhibitory effect on GvL.

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**CHRONIC GvHD HAS A ROLE IN MAINTAINING REMISSIONS AFTER IMATINIB IS DISCONTINUED IN Ph+ ALL PATIENTS TRANSPLANTED IN CR1**

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Backgrounds. Reappearance of BCR-ABL transcripts after allogeneic stem cell transplantation for Ph+ALL indicates evolving relapse. Intervention with imatinib may be associated with renewed and sustained PCR negativity. However, the clinical consequences of discontinuing imatinib are not known. Methods. We updated a previously reported prospective study of post-transplant imatinib for molecular relapse to assess the outcome of patients in whom imatinib was discontinued after sustained PCR negativity. Results. The present analysis includes exclusively the subset of 14 patients in whom BCR-ABL transcripts became undetectable shortly after (median: 46d, range: 27-111d) initiation of imatinib. Fourteen of 15 patients who did not achieve early PCR negativity have relapsed. When imatinib was discontinued after 12.2 (range: 1.4-17.5) months, BCR-ABL transcripts were undetectable in 15 of the 14 patients who had achieved PCR negativity. A median of 16.5 (range 3.3-39.4) months after stopping imatinib, 6 of these 14 patients were PCR negative and alive, one experienced reappearance of BCR-ABL transcripts and is currently treated with imatinib and donor lymphocyte infusions (DLI), 3 patients died in molecular CR, and 3 patients relapsed. Conclusions: We conclude that it is appropriate to discontinue imatinib in patients who previously underwent allogeneic SCT, remained PCR negative on imatinib for approximately one year, and have ongoing chronic GvHD. Absence of GvHD is associated with a higher risk of relapse following termination of imatinib therapy.

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**Platelets and bleeding disorders**

**1020**

**INCIDENCE, CLINICAL-LABORATORY FEATURES AND MANAGEMENT OF ACQUIRED VON WILLEBRAND SYNDROME AND OTHER ACQUIRED DEFECTS OF HEMOSTASIS IN A COHORT OF 240 PATIENTS WITH CHRONIC LYMPHO-MYELOPROLIFERATIVE DISORDERS**

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Background. Acquired von Willebrand Syndrome (AVWS) is a rare bleeding disorder with laboratory findings similar to those for congenital von Willebrand disease. The actual prevalence of AVWS in the general population is unknown because large prospective studies on this syndrome are not available. Retrospective data showed that AVWS is especially frequent in lympho- (LPD) or myeloproliferative (MPD) disorders. Aims and design of the study. To determine incidence, clinical-laboratory features and management of AVWS and other acquired hemostatic defects, we have sequentially observed for one year our cohort of patients with chronic LPD/MPD. Exclusion criteria were platelet counts <70,000/uL and any therapies, including non-steroidal anti-inflammatory drugs. Methods. A bleeding severity score derived from a detailed history of 11 symptoms. Screening tests: bleeding time (BT), prothrombin time (PT), partial thromboplastin time (PTT), thrombin time (TT) and, if prolonged, PT-PTT-TT 50:50 mixing tests. Additional specific tests: FVIII/VWF activities (AVWS/HA); platelet nucleotides (acquired storage pool defects, ASPD); silic clotting time (SCT), Russel viper venom time (RVVT), antidiopplid antibodies (ACA) for lupus anticoagulant-antiphospholipid antibodies (LAC/APA). Results. Among 458, 240 patients satisfied the inclusion criteria, with percentual (%) diagnosis of MGUS (58), ET (38), CLL (7), PV-CML-IM (7), HD-NHL (5), MDS M (2), MM (2) and amyloidosis (1). Results are:

<table>
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<th>Features</th>
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<tr>
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<td>118 (49)</td>
<td>240 (100)</td>
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<td>30/122 (25)</td>
<td>18/118 (15)</td>
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<td>3/118 (3)</td>
<td>11/240 (5)</td>
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<tr>
<td>4) anti FvIII or X inhibitors</td>
<td>3/122 (2)</td>
<td>4/118 (3)</td>
<td>7/240 (3)</td>
</tr>
</tbody>
</table>

In one year, severe mucosal (n=21) and non-mucosal (n=13) bleeds in LPD (n=12) or MPD (10) were treated with DDAVP (n=18), FFP/concentrates (n=4), IVIG (n=10), rFVIIa (n=2). Conclusions. AVWS and the other acquired hemostatic defects shown here are not so rare (9/16%) and can be severe in LPD/MPD. An early correct diagnosis should improve morbidity and mortality of patients with bleeding complications in chronic LPD/MPD.

**1021**

**DIFFERENT ALLELIC DISTRIBUTION OF SINGLE NUCLEOTIDE POLYMORPHISMS AT CODONS 10 AND 25 OF TGFß IN A GROUP OF 122 ITALIAN PATIENTS AND CONTROLS**

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Hereditary hemorrhagic telangiectasia (HHT) (OMIM #18700) is an autosomal dominant vascular disorder characterized by telangiectases on skin and mucosa (causing epistaxes and gastrointestinal bleeding, that may be severe enough to require transfusions) and visceral arteriovenous malformations (AVMs). Epistaxes and telangiectases are the most frequent symptoms, present in more than 95% of the patients. AVMs

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are mostly observed in liver, lungs and brain and may cause severe life-threatening complications. The phenotype is highly variable, even among members of the same family, and penetrance is usually complete by the age of 40 years. About 80% of HHT patients carry mutations in either of two genes: ENG (OMIM #131195) (HHT1) or ACVRL1 (OMIM #601284) (HHT2) which code for a TGFβ receptor type III and I respectively. Even if the primary locus has been identified, associated with HHT in whom the causing mutation in ENG or ACVRL1 was known. Methods. A 500 bp fragment of TGFβ gene including codons 10 and 25 was amplified by PCR and subsequently digested by MspAI (codon 10 SNP) and FseI (codon 25 SNP) enzymes. Digested products were analysed on polyacrylamide 7% and agarose 3% gels respectively. A subgroup of 20 patients was genotyped by PCR-SSP using the cytokine typing kit provided by Pel Freez Company. Statistical analysis was performed using the χ2 test (202 controls). Results. A statistically significant difference in the distribution of codon 10 and 25 was observed; for codon 10, it was limited to the subgroup carrying the ACVRL1 mutation, while the allele distribution at codon 25 was more widely different from controls. This skewed distribution leads to statistically significant differences in the TGFβ producer genotypes between controls and HHT patients: in this last group, in fact, there is a higher than expected percentage of intermediate and low producers (p<0.01). Summary: HHT is a vascular autosomal dominant disorder characterised by nosebleeding, telangiectasies and AVMS. A wide inter and intra-familial variability in the phenotype is present. The genes involved (ENG and ACVRL1) belong to the TGFβ signalling pathway and we assessed genotype distribution of two TGFβ SNPs (codons 10 and 25) related to the protein production in a group of 122 HHT patients with a known causing mutation. Statistically significant results were obtained for codon 25 and some codon 10 subgroups. We suggested that differences in TGFβ production can partially explain the phenotypic variations.

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ELTROMBOPOAG INCREASES PLATELETS DURING 6-WEEK TREATMENT OF ITT RESULTS OF A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED PHASE II STUDY

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Background/Annex. Ertrombopag olamine, a novel, small molecule, oral platelet growth factor was studied in a global, randomized, double-blind, placebo-controlled phase II trial in adult patients with chronic idiopathic thrombocytopenic purpura (ITP) and platelet counts <30,000/µL. Methods. The primary efficacy endpoint was the proportion of patients with platelets greater than or equal to 50,000/microL after 42 days of dosing using last observation carried forward. Randomization was stratified by spleenectomy status, use of concomitant ITP therapy and platelet counts less than or equal to 15,000/µL. Results. One hundred and four patients were randomized into placebo (N=26), 50µg (N=27), 50µg (N=27) and 75µg (N=24) ertrombopag arms. The majority of patients were females (62%) and of Caucasian origin (70%). Prior treatment of ITP included corticosteroids (73%), IVIG (57%) and anti-D (27%). During the study 35 (34%) patients received concomitant ITP therapy. At Day 45, a dose dependent increase in the proportion of responders (platelet count greater than or equal to 50,000/µL) was observed: placebo (16%), 30µg (28%), 50µg (67%) and 75µg (86%). The odds-ratio of treatment response to placebo was statistically significant in the 50 and 75µg arms (p<0.001). Similar efficacy response of ertrombopag relative to placebo was observed regardless of strata (spleenectomy status, concomitant ITP treatment and baseline platelet count). The percentage of patients achieving a platelet count >200,000/µL was: placebo (4%), 30µg (12%), 50µg (38%) and 75µg (52%). The median platelet count on Day 45 was 16, 29, 152 and 202,000/µL for placebo, 30µg, 50µg and 75µg ertrombopag, respectively. Overall, the safety profile was similar across the treatment groups, with the following percentage of patients experiencing at least one adverse event (AE) during treatment: placebo (58%), 30µg (44%), 50µg (44%) and 75µg (58%). Headache, AST elevation, conjestion and epistaxis were the only AEs occurring in greater than or equal to 5% of patients in the ertrombopag arms. The most common AE in the ertrombopag 75µg arm was fatigue, occurring in 19% of patients, compared to 3% of all patients exposed to ertrombopag. The most common AE in the ertrombopag 75µg arm was headache (21%), compared to 15% of patients exposed to placebo. A total of 2(8%) placebo and 2(7%) 50µg patients experienced at least one serious AE (SAE) during treatment; of which, 1(4%) placebo patient and 1(4%) 50 µg patient experienced at least one related SAE during treatment. No SAEs were reported during the treatment period on the 30 µg and 75µg ertrombopag arms. No other dose dependent safety concerns were identified. Summary/Conclusions. Ertrombopag at doses of 30 and 75µg significantly increased platelet counts during the 6 week treatment period compared to placebo. No dose dependent safety concerns were identified. Phase III trials of ertrombopag in patients with ITP are ongoing.

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GENETIC DEFECTS CAUSING TYPE 3 VON WILLEBRAND DISEASE IN HUNGARY

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Background. Type 3 VWD, an autosomal recessive severe bleeding disorder, is characterized by very low to undetectable plasma von Willebrand factor (VWF) and, consequently, reduced plasma factor VIII levels. Genetic mutations responsible for type 3 VWD are very heterogenic, and show variability among different patient populations. With the exception of 2453delC, repeatedly found in populations adjacent to the Baltic Sea, no prevalent mutation has been found in populations so far studied. Aims. We set out to characterize the genotype of type 3 VWD in the entire Hungarian population of ten million. Methods. We studied the genetics of 27 patients by direct sequencing of all 52 exons of the von Willebrand factor gene. Results. The prevalence of the disease in Hungary is 0.29/100.000. Several novel nonsense and frame shift mutations were identified: Q1238*, Q1198*, Q1931*, S2566*, S2505* and 1995insNC, 214delCT, 3622delT, S516insGA, 7383delIG. Previously described mutations found in Hungarian patients were: R1659*, R1853* and 2209delCT. In addition, a large partial deletion at the 5'-end of the gene was also identified. 2453delC in exon 18, the single most frequent deletion in other populations of Europe, was detected in 6 patients (allele frequency among patients approximately 18%). Finally, three novel missense mutations and two possible splice site mutations were also detected: CS5R, R81G, C623T and S2979+1G→A (reported previously), 1700-10C→A. Summary. Type 3 VWD is caused by heterogeneous mutations scattered throughout the entire gene. If confirmed by a detailed comparison to other populations, the above new mutations suggest that the Hungarian type 3 VWD patient population may be genetically distinct.
The low molecular weight TPOR agonist, eltrombopag, does not prime platelet activation in vitro

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Backgrounds. Eltrombopag is a non-peptidyl small molecule thrombopoietin receptor (TPOR) agonist currently in clinical development for the treatment of patients with thrombocytopenia. Stimulation of this receptor results in enhanced megakaryocyte proliferation, differentiation, and ultimately platelet production. In addition to effects on megakaryocytes, TPOR activation via thrombopoietin (TPO) is known to directly stimulate platelet function. The physiological consequences of platelet stimulation in this setting are unclear; however, it could represent a general liability in the utilization of TPOR agonists. Aims. The objective of the present study was to compare the direct platelet activating potential of eltrombopag to TPO in vitro. Methods. Platelets were obtained from healthy volunteers, and the activation of signal transduction pathways was examined in washed platelet preparations. Platelet aggregation was examined under multiple experimental conditions, including washed platelet preparations and platelet-rich plasma (PRP) anticoagulated with either citrate or hirudin. Platelet α-granule release was determined via FACS measurement of CD62P. Results. In signal transduction studies of washed human platelets, TPO activated Stats-1,5,5 and Akt. In comparison, eltrombopag partially activated Stats-3 and 5, with no/minimal activation of Stat-1 or Akt. In platelet aggregation studies, TPO acted in synergy with subthreshold/submaximal concentrations of ADP or collagen to induce maximal aggregation under all conditions examined. In contrast, eltrombopag induced weak and inconsistent activation of washed platelets; however, no synergy was observed when examined in PRP. Similar to aggregation results, platelet activation as examined via surface expression of CD62P was significantly enhanced by TPO as compared to eltrombopag. Conclusions. The present study demonstrates that the TPOR agonist eltrombopag has only a limited capacity to induce human platelet activation, suggesting that potential platelet activation liabilities associated with peptidyl TPOR agonists could be attenuated via a small molecule approach.
A FLOW-CYTOMETRIC IMMUNOBEAD ASSAY FOR THE DETECTION OF BCR-ABL FUSION ONCOPROTEIN IN PRECURSOR-B-ALL

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The BCR-ABL fusion gene, caused by t(9;22), is a frequent chromosomal aberration in precursor-B-ALL patients (25%-40% of adults cases, 5%-5% of pediatric cases) and is associated with a poor prognosis, requiring a high-intensity treatment protocol. Rapid detection of the BCR-ABL translocation in precursor-B-ALL patients at diagnosis is therefore important for the choice of treatment. Current approaches for the detection of the BCR-ABL fusion gene employ karyotyping, FISH or PCR. However, these techniques take relatively long and demand a specialized laboratory. Our aim was to develop a flow cytometric bead-based assay (CBA) that detects the BCR-ABL fusion gene product in an easy, rapid and accurate manner. In this assay, a bead-bound catching antibody recognizes one part of the fusion protein, whereas a biotin-conjugated detection antibody recognizes the opposite part of the fusion protein. Only when a lysate of patient cells contains the fusion protein, immunobeads will give a positive signal in the flow cytometer. We generated a novel antibody, specifically recognizing the exon 1-encoded domain of the BCR protein, using a developmental strategy and screening method that increases the likelihood of producing antibodies that are suitable for flow cytometry. The anti-BCR antibody was coupled to BD CBA Flex beads. After incubation of these beads with cell lysate, an already existing biotinylated anti-ABL antibody was used as detection antibody together with streptavidin-PE. Using this bead assay, we detected a strong PE signal in cell lysates of the cell lines K562, LAMA-84 (both p210), TOM-1 (p190) and AR230 (p230) harboring the BCR-ABL translocation, but not in cell lines with other translocations or normal PBMCs. A robust signal could be detected with less than 1% of LAMA-84 cells in a background of normal PBMCs, showing the high sensitivity of the assay. We then tested the assay on lysates of precursor-B-ALL patients and showed a highly specific signal only in patients that were tested positive for the BCR-ABL fusion gene using standard PCR and/or FISH techniques. We conclude that this novel bead assay can be used for diagnosis of precursor-B-ALL patients. The new assay has major advantages over currently used techniques. It is fast (completed within a few hours) and can easily be performed in a standard diagnostic laboratory using routine flow cytometry. The assay is accurate and sensitive and as recognition does not involve the break-point region, all different BCR-ABL protein variants (p190, p210 and p230) can be detected. Furthermore, as only a minority of patients treated with imatinib achieve a molecular remission, this bead assay will be of decisive value for the careful assessment during the first 6 months of treatment.
DELETIONS OF DERIVATIVE CHROMOSOME 9 IN PATIENTS WITH CHRONIC MYELOGENOUS LEUKAEMIA: ONLY DELETIONS THAT SPAN THE ABL/BCR BREAKPOINT ARE ASSOCIATED WITH ADVERSE PROGNOSIS

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Deletions at the ABL-BCR reciprocal breakpoint on the derivative chromosome 9 are seen in 10-15% of patients with CML and have been associated with a poor prognosis, at least for cases treated with hydroxyurea or interferon α (IFN). Studies to date have used different FISH probe sets to determine deletion status and thus the results are not always directly comparable. Furthermore, information concerning the extent of deletions is limited. To provide more accurate information about deletion status, we have developed a rapid DNA-based screen based on multiplex ligation-dependent probe amplification (MLPA). Probes were designed to the deleted region both the upstream and downstream of the breakpoint, plus several control loci. MLPA was performed using standard conditions and peak heights were determined using an ABL 3100 Genetic Analyzer and Genotyper software. Since patient samples may contain a variable proportion of normal cells, we determined the sensitivity of the assay to detect the der(9) deletion in MC3 cells. We found that the deletion was readily detectable in dilutions of MC3 DNA in normal DNA at a level of 60% or greater, indicating that the assay was applicable to the great majority of CML patients. We then went on to perform a retrospective study of 348 patients (204 male; 144 female; median age 50 years, range 11-85) who had been enrolled into the German CML I, II or III studies between 1987 and 1999. This represents the largest study on the prognosis of der(9) deletions to date. All patients received IFN as first line therapy but 61 were subsequently treated with imatinib and 138 subsequently underwent stem cell transplantation (SCT). At the time of analysis, 161 patients were still alive at a median of 8.8 years (range 2.6-16.5). MLPA was performed on samples taken prior to treatment and showed that 59/348 (16.9%) of cases had der(9) deletions, defined as loss of at least two consecutive markers. Unexpectedly, we found that patients with deletions survived longer than those without deletions, although the difference was not significant (9.8 versus 7.3 years; p=0.17). This effect was seen both for cases that underwent SCT (not deleted: n=116, median = 9.4 years versus deleted: n=22, median not reached; p=0.34) or did not undergo SCT (not deleted: n=173, median = 6.8 years versus deleted: n=87, median = 9.8 years; p=0.27). However, the 21 cases who had deletions that spanned the translocation breakpoint did show inferior overall survival when compared to all other cases (5.7 versus 8.8 years; p=0.0025). This was not seen for patients with deletions on the ABL side only (n=20) or the BCR side only (n=18) and in fact both these groups showed longer survival compared to all other patients. In conclusion, MLPA is a reliable technique for detection of der(9) deletions in CML. Our analysis indicates that only those deletions that extend to both sides of the reciprocal ABL-BCR fusion breakpoint are associated with adverse prognosis.

MYELODYSPLASTIC SYNDROMES


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The myelodysplastic syndromes (MDS) are a heterogeneous group of hematopoietic malignancies characterized by blood cytopenias, ineffective hematopoiesis and hypercellular bone marrow. We have used Affymetrix microarray technology to determine the gene expression profiles in CD34+ cells of MDS patients and controls. Fifty-five MDS patients (18 RA, 19 RARS and 18 RAEB) and 11 controls were included in the study. Twenty of 55 patients had a del(5q). CD34+ cells were isolated from bone marrow samples of patients and controls using MACS magnetic columns. Extracted total RNA was amplified using the Two-Cycle Target Labelling kit (Affymetrix). Biotin-labelled fragmented cRNA was hybridized to Affymetrix U133 Plus2.0 chips (47,000 transcripts, representing 39,000 human genes). Cell intensity calculation and scaling was performed using GeneChip Operating Software and data analysis using GeneSpring 7.2. The expression profiles of MDS CD34+ cells showed many similarities to reported interferon-α induced gene expression in normal CD34+ cells. Indeed the two most up-regulated genes, IFIT1 and IFITM1, are interferon stimulated genes (ISG). IFITM1 and IFIT1 were up-regulated by >2-fold in 37/55 MDS patients and by >10-fold in many cases. Genes down-regulated by >2-fold in the majority of MDS patients include the putative tumor suppressor gene Gravin/AKAP12, ARPP-21, CD24 and MME. Gravin/AKAP12 was down-regulated in 44/55 MDS patients. The results for several genes have been confirmed by real-time quantitative PCR (TaqMan). The association of distinct gene expression profiles with specific FAB and cytogenetic groups was determined. Hierarchical clustering performed using a set of 457 significantly different genes showed that MDS patients with RARS constitute a homogeneous group, while MDS patients with RA and RAEB show more overlap. CD34+ cells from MDS patients with RARS showed up-regulation of mitochondrial-related genes, and in particular of those of heme synthesis (e.g. ALAS2). Statistical analysis showed that 889 probe-sets could discriminate MDS patients with a del(5q) from those without a del(5q). Approximately 40% of the 889 probe sets mapped to chromosome 5q and their expression levels were lower in MDS patients with del(5q) than those without del(5q), suggesting that the deletion in patients with a del(5q) has a gene dosage effect. MDS patients with the del(5q) showed distinctive up-regulation of the histone HIST1 gene cluster at chromosome 6p21 and of genes related to the actin cytoskeleton. In order to identify genes differentially expressed between early and advanced MDS, a comparison was made between the 18 patients with RA and the nine MDS patients with RAEB-II. 762 significantly different probe sets were identified that could group together MDS patients with RAEBII. The most significant genes identified include CASP3 and FLT3, and represent potential prognostic markers or markers of disease progression. This study provides important and new insights into the pathophysiology of MDS.

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LENALIDOMIDE SELECTIVELY INHIBITS IN VITRO GROWTH OF THE MALIGNANT CLONE AND UP-REGULATES SPARC IN MYELODYSPLASTIC SYNDROME (MDS) PATIENTS WITH 5q DELETION


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Backgrounds. In a phase II trial the immunomodulatory drug lenalidomide induced 75% complete cytogenetic remissions in patients with
myelodysplastic syndrome (MDS) and 5q-1 deletion. Lenalidomide has been shown to inhibit angiogenesis, cell adhesion, and secretion of TNF-α, and to modulate other cytokines. Furthermore, lenalidomide stimulates T-cells and NK-cells, and directly induces apoptosis in myeloma cells. It is not well characterized how these effects are mediated, nor which effects that are central for the impact on cancer cells. Aim: To assess the direct effects of lenalidomide on growth, differentiation, and gene expression of hematopoietic cells from MDS patients with del(5q)(q31) and healthy controls. Methods: Selected CD34+ hematopoietic progenitors from 12 MDS patients with del(5q)(q31) and from 10 healthy controls were cultured with or without 10 μM of lenalidomide in a 14-day model for erythroblast differentiation (with medium containing IL-3, IL-6, and SCF; with addition of Epo during the second week). FISH and FACS analyses were performed at day 0, 7, and 14. The median proportion of 5q- cells by FISH at day 7 was 98% (range 86-99), dropping to 84% (range 14-98) at day 14 due to a variable outgrowth of cytokinetically normal cells. Gene expression profiling was performed on day 7 cells from 6 MDS patients and 5 healthy controls using Affymetrix Human Genome U133 Plus 2.0 Arrays. Results. In erythroblasts cultures with cells from healthy controls, lenalidomide had no inhibitory effect on fold increase of cell counts (p>0.60). However, in cultures with cells from 5q- patients, the clone with 5q deletion showed significant inhibition of fold increase at day 14 (p=0.04), while the cytokinetically normal progenitors were not inhibited (p=0.00). FACS analysis at day 14 showed that lenalidomide samples had higher proportions of cells expressing erythroid markers and lower proportions expressing myeloid markers. Gene expression profiling showed that four genes were up-regulated by lenalidomide in all MDS 5q- control samples analyzed: Z33C, LRP11, and SPARC. The median up-regulation of SPARC was 4.3-fold (range 2.3-9.4). LRP11 and HBA2 were down-regulated in 10 of 11 samples. Several of the differentially expressed genes warrant further investigation. The SPARC gene has been postulated to be a tumor suppressor gene in AML and has been shown to have anti-proliferative and anti-angiogenic effects. Interestingly, the SPARC gene maps within the commonly deleted region (CDR) of the 5q- syndrome at 5g31-q52. Conclusions. Lenalidomide selectively inhibits in vitro growth of 5q-hematopoietic progenitors, while not affecting growth of cytokinetically normal cells from MDS patients with 5q deletion or from healthy controls. In addition, lenalidomide affects cell differentiation and induces changes in gene expression, including up-regulation of SPARC. We hypothesize that one part of the potent effects of lenalidomide is mediated through increased SPARC expression. Whether the localization of the SPARC gene to the CDR of the 5q- syndrome is significant or not to the pathogenesis of the 5q- syndrome remains to be determined.

Clinical benefit from 2 phase II trials evaluating lenalidomide (revlimid) in lower-risk myelodysplastic syndrome patients with or without del 5q.

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Transfusion-dependent myelodysplastic syndrome (MDS) is a serious illness which adversely impacts overall survival. Results from 2 phase II clinical trials have shown that oral lenalidomide produces meaningful hematological improvement in patients with low- or intermediate-1-risk MDS with or without an associated del 5q cytogenetic abnormality (A. List et al EHA 2005, A. Raza et al. MDS 2005). To investigate possible differences in efficacy and safety of lenalidomide in lower-risk MDS patients with or without an associated del 5q cytogenetic abnormality. Results: From patients from 2 phase II clinical trials (MDS-002, MDS-005) of lenalidomide were analyzed. Differences in the frequency of red blood cell (RBC)-transfusion independence, improvement in hemoglobin, cytogenetic and pathologic response and safety were evaluated. In the del 5q patients, 67% (66/97) of the patients who had become RBC-transfusion independent remained RBC-transfusion independent. The duration of response was at least 24 weeks in 84% (83/97) of the responders and was at least 52 weeks in 52% (52/97) of the patients. The median increase in blood Hb from baseline to the maximum Hb achieved during RBC-transfusion independence was 5.5 g/dl (range, 1.1 11.4 g/dl, n = 99). Major cytogenetic responses were observed in 44% (32/72) and minor cytogenetic responses were observed in 29% (21/72) of the patients who were evaluable for cytogenetic response. Among patients with available follow-up bone marrow aspirate specimens, the follow-up bone marrow aspirates from 33% (27/81) of the patients had no evidence of MDS. In the non del 5q population, 26% (56/215) of the patients had achieved RBC-transfusion independence by ITT analysis during lenalidomide therapy. In this population, 77% had a normal karyotype and no difference was observed in transfusion independence rate between patients with a normal versus abnormal karyotype (29% and 27%, respectively). The duration of response was at least 24 weeks in 17% (36/215) of the responders and was at least 52 weeks in 10% (22/215) of the patients. Lenalidomide-induced transfusion independence was associated with a median increase from baseline in Hb of 3.0 g/dl in the responders. Neutropenia and thrombocytopenia were the most common adverse events and were reported at least once in 44% (172 and 174/395, respectively) of the patients who were treated with the 10 mg/day starting dose. Combined disease- and treatment-associated mortality (6%; 25/408) was relatively low and appeared consistent with the survival reported for lower-risk MDS. Lenalidomide is an effective and well-tolerated treatment for a select group of patients with low- or intermediate-1-risk MDS without an associated del 5q cytogenetic abnormality. From these data it becomes evident that there is a subpopulation of the non del 5q patients who respond similarly to patients with del(5q) and to other lower-risk MDS. These studies are warranted to investigate pathogenetic differences that account for the karyotype dependence in the frequency and durability of response.

EFFECT OF LENALIDOMIDE (CC-5013) ON GENE EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) IN MYELODYSPLASTIC SYNDROME WITH DEL(5Q) CHROMOSOME ABNORMALITY AND ITS RELEVANCE TO ANGIOGENESIS IN BONE MARROW

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Background. Lenalidomide, a novel immunomodulatory drug (IIMD), is a potent antiproliferative and antiangiogenic agent. Vascular endothelial growth factor (VEGF) and its receptor (KDR) are major regulators of angiogenesis, which plays a key role in the growth and progression of solid tumors and hematologic neoplasms, and increased plasma levels of KDR were correlated with a lower remission rate in patients with myelodysplastic syndromes (MDS). Methods. From a total of 35 patients suffering from MDS with or without del(5q) and being treated within a single-center trial on the efficacy of CC-5013, biopsies and aspirates from bone marrow taken before and 6+ 12 months after start of lenalidomide therapy were evaluated for the percentage of cells with del(5q), blast count, vascularization, and gene expression of VEGF and its receptor using cytologic, histopathologic, cytogenetic and molecular genetic methods. 20 patients with healthy marrow served as a control for normal marrow. Results. Before start of treatment, vascularity of bone marrow, measured as the total length of vessels within the marrow volume, was markedly increased in MDS exceeding that from normal marrow by a factor of 3.24 ± 2.57 (p<0.00005). Increase of vascularity correlated with increase of VEGF expression, which exceeded that from normal marrow by a factor of 2.68 ± 2.21 (p = 0.0005), whereas KDR gene expression was not significantly changed. During therapy with lenalidomide, vascularity markedly decreased to normal values in 60% of patients (21 / 85; p< 0.00005). Normalization of vascularization correlated with a major cytogenetic response of disease (> 35% metaphases with del(5q), 0.00005) which occurred in 21 patients, 12 of them with a complete cytogenetic remission. Anti-angiogenic inefficacy of lenalidomide correlated with progression of disease (>= 5% blasts in blood or bone marrow; 14 patients; p=0.0005). In contrast to vascularity, VEGF and KDR gene expression were not reduced during lenalidomide treatment, the correlation between vascularity and VEGF gene expression disappeared (p< 0.00005). Conclusions. In MDS with del(5q), lenalidomide uncouples angiogenesis from the effect of VEGF resulting in a significant reduction of marrow vascularity followed by an increase in gene expression of
Most mammalian genes undergo alternative pre-mRNA splicing, which generates diverse transcripts and protein isoforms that may be regulated in a tissue- or developmental stage-specific manner. Aberrant and alternative splicing may be associated with neoplasia, and can contribute to alterations in gene expression detectable by oligonucleotide microarrays, though the function of the novel spliceforms in cancer pathogenesis is, for the most part, unknown. While studying ATRX, an X-linked gene encoding a chromatin-associated transcriptional regulator that was recently shown to be mutated in patients with MDS and an acquired thalassemic phenotype (Gibbons R et al. Nature Genetics 2003 and Steensma DP et al. Blood 2004), we discovered a novel exon-skipping and frameshifting alternative spliceform in the region of the gene that encodes the conserved helicase domain of the protein. A cis-acting genomic DNA mutation of ATRX was not detected, leading us to hypothesize that aberrant splicing as a consequence of trans-acting defects altering basal splicing machinery or regulators of alternative splicing might be common in MDS. To further characterize aberrant/alternative splicing of ATRX and other representative genes in MDS. We performed RT-PCR of marrow and blood cells from patients and healthy controls of ATRX, CDC25C (a gene at 5q31.1 that encodes a phosphatase important in cell cycle regulation, with expression that changes during lenalidomide therapy), and H3F3A/LSH/PASG/SMARCA6 (encodes a SWI/SNF2-related helicase that, like ATRX, localized to pericentromeric heterochromatin). Amplicons were analyzed by DNA sizing column chromatography under non-denaturing conditions, cloned into DH5α competent cells using the pGEM-T Easy system, and sequenced. The novel aberrant ATRX exon-skipping transcript was not present in 20 varieties of normal tissue from autopsies (gut epithelium, testis, myocardium, etc.), and was detected in blood cells from only 1 of 24 healthy volunteers (transiently). In contrast 7/13 patients with MDS and 4/16 patients with myeloid leukemia exhibited the ATRX variant in hematopoietic cells, in some patients in equal or greater proportion than the normal transcript. In MDS patients treated with chemotherapy who achieved a cytogenetic remission, the aberrant ATRX transcript disappeared, and was again detectable at the time of disease relapse. We also observed a series of novel exon-skipping or intron-retaining alternative spliceforms of CDC25C that were not present in healthy controls, and splicing patterns of H3F3A/LSH/PASG/SMARCA6 were likewise disrupted in MDS primary samples. A subset of patients with MDS may exhibit a generalized defect in pre-mRNA splicing that leads to the generation of aberrantly spliced isoforms in multiple genes of potential pathobiologic relevance. The etiology and functional significance of these variants should be explored further. Because the nature of neoplasia-associated alternative gene products is often consistent with an active role in cancer, this suggests a potential therapeutic target in malignancies such as MDS that display aberrant splicing.

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AN ABBERRANT MRNA SPLICING PHENOTYPE IN MYELODYSPLASTIC SYNDROMES (MDS)

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VEGF and KDR. Inhibition of angiogenesis correlates with a significant reduction of the MDS clone in bone marrow whereas increase of vascularization indicates progression of disease.

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ANTIBODIES IN THE TREATMENT OF CHRONIC LYMPHOCYTIC LEUKAEMIA

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A high incidence of unexplained skin rashes and auto-GvHD was observed after an alemtuzumab containing myeloablative conditioning regimen and autologous stem cell transplantation (SCT) in patients with CLL. 1) Comparison of CLL patients undergoing autologous stem cell transplantation after conditioning with TBI/Cy ± Alemtuzumab. 2) Detailed analysis of the defects of immune reconstitution. 3) Analysis of the influence of auto-GvHD on minimal residual disease. Methods. This study treated 27 patients with CLL (Binet B / C) with autologous SCT in two trials of the German CLL Study Group at the University of Ulm (CLL3 & CLL3C trials). Patients received cytoreduction with fludarabine plus cyclophosphamide and stem cell mobilization with Dexa-BEAM. In the CLL3 trial (n=11) autologous SCT was performed after standard conditioning with 12 Gy total body irradiation and cyclophosphamide (120 mg/kg) (TBI/Cy). Patients in the CLL3C trial (n=16) were treated identically except for the addition of alemtuzumab before SCT (mean 100 mg iv) (Alem/TBI/Cy). There were no skin rashes or auto-GvHD in the standard TBI/Cy group. In contrast, 12 of 16 patients (75%) receiving Alemt/TBI/Cy developed a skin rash (maculopapular rash (n=5), erythrodemia (n=3), eczema (3)) between 48 and 801 days after SCT. In 7 patients a clinical diagnosis of auto-GvHD was made. Typically, concurrent symptoms at the onset of auto-GvHD included conjunctivitis (n=4), sicca syndrome (n=5), and cholestasis (n=4). The histological findings were compatible with GvHD grade 1-2 in all five patients with clinical auto-GvHD in whom skin biopsies were performed. The median duration of GvHD was 517 days (range 60-867) and the reduction of immunosuppression led to a flare of the skin rash in 5 of 7 patients. The reconstitution of CD4 and CD8 positive cells was severely delayed in the Alem/TBI/Cy group with a particular depletion of CD8+ cells for up to 2 years. The CD4/CD8 ratio was abnormally high in the Alem/TBI/Cy group. This increased ratio was mainly caused by the extreme CD4/8 ratio imbalance in patients with GvHD. The CD4/8 ratio was 20 times higher among patients with auto-GvHD as compared to patients without GvHD (all time points combined). Interestingly, histology showed a predominant invasion of the skin by CD4 positive T lymphocytes. Molecular analysis revealed oligoclonal expansion of T cells in the skin biopsy samples of patients with GvHD with similar Vβ subfamilies (BV5, BV18)(n=3). The addition of alemtuzumab led to continuous MRD negativity in 10/14 patients (71%) compared to 0/7 patients receiving TBI/Cy (p=0.0039). Within the Alem/TBI/Cy group continuous MRD negativity was observed in 6/6 patients with auto-GvHD (100%) vs. 4/8 patients without auto-GvHD (50%; p=0.08). The current study demonstrates a remarkable incidence of a GvHD-like syndrome attributable to the addition of alemtuzumab to the TBI/Cy conditioning regimen before autologous SCT in patients with CLL. The addition of alemtuzumab to the conditioning regimen led to improved disease control at the molecular level and longer follow-up will show if the GvHD-like syndrome will lead to an anti-leukaemia effect and prolonged MRD negativity.
Chlorambucil (CHLO) is an approved therapy for patients with B-CLL. Alemtuzumab (CAM) has suggested effectiveness in untreated and demonstrated efficacy in relapsed and refractory B-CLL. CAM307 is an international, randomized, open-label study comparing efficacy and safety of CAM versus CHLO in previously untreated patients with B-CLL. Presenters are the preliminary safety and efficacy results of this study. Eligible pts were Rai stage I-IV with evidence of progression requiring therapy. Patients with secondary malignancies, autoimmune thrombocytopenia, active infection, central nervous system involvement, or who were positive for cytomegalovirus (CMV) via quantitative PCR, were excluded from the trial. Patients were randomized 1:1 to receive either CAM 50 mg IV 5 times a week for up to 12 weeks or CHLO 40 mg/m² PO on day one of a 28-day cycle for up to 12 cycles. All patients in the CAM arm received prophylaxis with trimethoprim/sulfamethoxazole DS and foscarnet during therapy and until CD4+ counts returned to ≥ 200 cells/µL. Accrual completed with 297 patients (213 males, 84 females; median age 60 yrs [range: 55-86]); CAM n=149 and CHLO n=148. Treatment arms were well balanced for key prognostic factors analyzed to date; overall, 96% were WHO PS 0-1, 70% had < 5 cm lymphadenopathy. Median length of treatment was 11.7 wks for CAM and 24.4 wks for CHLO. An independent analysis of response showed an 82.6% Overall Response (OR) in the CAM arm compared to 54.7% OR in CHLO (p<0.0001), and patients in the CAM arm had a significantly higher CR rate compared to those in the CHLO arm: 22.1% vs 2.0% (p=0.0001). Preliminary analysis of the pertinent safety data reported through October 24, 2005 is summarized in the Table.

### Table 1. Phase III CAM study.

<table>
<thead>
<tr>
<th>Safety</th>
<th>CAM</th>
<th>CHLO</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 3/4 events</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>17.7%</td>
<td>15.6%</td>
<td>0.7546</td>
</tr>
<tr>
<td>Anemia</td>
<td>12.2%</td>
<td>15.0%</td>
<td>0.6103</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>42.2%</td>
<td>23.1%</td>
<td>0.0007</td>
</tr>
<tr>
<td>Febrile neutropenia</td>
<td>4.8%</td>
<td>3.4%</td>
<td>0.7697</td>
</tr>
<tr>
<td>Infection (excluding CMV)</td>
<td>14.3%</td>
<td>6.8%</td>
<td>0.0562</td>
</tr>
<tr>
<td>CMV infection</td>
<td>6.8%</td>
<td>0.0%</td>
<td>0.0017</td>
</tr>
</tbody>
</table>

*Comparisons were made using the Exact method.

Overall safety data indicate 34.7% of CAM patients and 19.7% of CHLO patients experienced a serious adverse event, with 21.1% and 4.1% considered drug related, respectively. One treatment related death occurred in the CHLO arm and none in the CAM arm. Infusion related events (fever, rigors, nausea, vomiting, and hypotension) were the most frequently reported CAM related events; however the severity was predominately grade 1/2 whereby only 13.6% of patients experienced a grade 3/4 event. The only pertinent grade 3/4 safety signals which attained statistical significance were neutropenia and CMV infection. As expected, the incidence of infection in the CAM arm was higher than the CHLO arm. However, there was no difference in the incidence of febrile neutropenia in the two study arms. Preliminary analysis of this randomized controlled trial shows that CAM had an OR and CR rate statistically superior to CHLO with manageable toxicity.
LUMILIXIMAB IN COMBINATION WITH FLUDARABINE, CYCLOPHOSPHAMIDE, AND RITUXIMAB (FCR) FOR PATIENTS WITH RELAPSED CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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Aim. A recently completed phase 1, single-agent study with lumiliximab showed evidence of clinical activity and a favorable safety profile. Preliminary data suggesting synergy with both fludarabine and rituximab resulted in initiation of a combination study of lumiliximab with FCR in previously treated patients with CLL. Aims. The objectives of this study are to determine the safety profile of lumiliximab in combination with FCR, recommend a phase 2 dose, and evaluate the clinical activity. Patients 18 years of age or older with relapsed CD23+ B-cell CLL were eligible for this open-label, dose-escalation, phase 1/2 study. For the phase 1 portion of the study, patients received either 375 mg/m² or 500 mg/m² of lumiliximab in combination with each 28-day cycle of FCR for 6 cycles. Results. No dose-limiting toxicity was noted in the phase 1 portion of this study (375 mg/m², n=3, and 500 mg/m², n=6). Grade 3 or 4 adverse events. Events reported in more than 1 patient were neutropenia (7 patients), leukopenia (4 patients), skin reactions (3 patients), thrombocytopenia (2 patients), and pyrexia (2 patients). Hematologic toxicity is comparable with the FCR regimen. Sixteen patients completed ≥3 cycles of treatment and 4 patients completed ≥2 cycles. Response was evaluated using NCI-WG criteria. Nine (45%) patients have confirmed complete responses, 5 (25%) have partial responses, and 6 (30%) patients have disease progression. Summary. Conclusion. Lumiliximab in combination with FCR is well tolerated and has shown no increased toxicity compared with FCR alone. Lumiliximab may enhance the CR rate with FCR when used for treatment of patients with progressive CLL after prior therapy.

ALEMTUZUMAB PLUS FLUDARABINE IN PATIENTS WITH FLUDARABINE REFRACTORY CLL (ON BEHALF OF THE NCRI CLL TRIALS SUB-GROUP)

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Patients with fludarabine refractory CLL have a median survival of 10 months with conventional chemotherapy. Intravenous (IV) alemtuzumab is approved in fludarabine refractory CLL resulting in 33 to 50% responses. Combined alemtuzumab and fludarabine can induce responses in CLL refractory to both agents. Infusion reactions and 2-hour infusions 3x a week for 12 weeks are problems with IV alemtuzumab. Subcutaneous (SC) alemtuzumab is more convenient but pharmacokinetics suggest the need for prolonged therapy with little efficacy data in fludarabine-refractory CLL. A study to assess the safety and effectiveness of SC alemtuzumab in fludarabine-refractory CLL. Methods. SC alemtuzumab was given at a dose of 30 mg 3x a week (after dose escalation) for up to 24 weeks depending on 6-weekly marrow assessments. Patients failing to respond to alemtuzumab in the trial could receive oral fludarabine (40 mg/m²/day for 3 days every 4 weeks) combined with SC alemtuzumab. In this planned interim analysis of the first 44 patients (median age 66, range 41 to 79) 2 patients died before receiving alemtuzumab, and 5 remain on therapy. Of the remaining 37 patients, one withdrew consent and 36 patients have completed therapy. Responses to alemtuzumab monotherapy (n=36) were 2 MRD negative CR, 1 MRD positive CR, 11 PR (including 1 MRD negative patient who remained cytopenic), 20 NR, and 2 died. Alemtuzumab was given for a median 12 weeks (range: 2-24) with a median dose of 913 mg (range 106 to 2173 mg). 12 patients (8 NR and 4 PR) received concurrent fludarabine and SC alemtuzumab (median 2.5 courses fludarabine [range 1-3]). Two non-responders achieved a PR and one of the partial responders achieved a CR (MRD positive). Therefore the overall response rate for the whole cohort was 16/36 (44%) including 3 MRD negative patients (2 CRs and 1 PR). IgVH gene was unmutated (>98% homology to germ line DNA) in 11/14. HSH revealed poor risk deletions (11q and/or 17p) in 21/34 patients (17p< in 9; 11q< in 6 and both in 6). p53 functional analysis is available for 23. 20/23 had p53/ATM dysfunction or deletion. 13/25 (52%) of patients with poor risk deletions (11q and/or 17p) or p53 dysfunction responded to therapy. The initial alemtuzumab dose was associated with localised erythematous skin reactions in 20 patients (diameter 1 to 18 cm), fever in 7 and rigors in 3. All reactions subsided in 48h. Serious infections during alemtuzumab monotherapy were: CMV reactivation (10); febrile neutropenia (9); invasive fungal infection (8); pneumonia (2). On the combination, CMV reactivation in 2 cases but no other grade 3+ infections. All CMV reactivations resolved on antiviral therapy. Grade 3+ thrombocytopenia and neutropenia was seen in 16 and 25 patients on alemtuzumab monotherapy as well as in 1 and 2 patients on combined therapy, respectively. We report that subcutaneous alemtuzumab is effective in poor-risk fludarabine-refractory CLL and is well tolerated compared to IV therapy. A longer duration of SC alemtuzumab therapy (up to 24 weeks) is required. The addition of oral fludarabine improves the response rates with acceptable toxicity.
Clinical studies in Non-Hodgkin's Lymphoma

**1040**
SURVIVAL IMPACT OF THE TIME REPEATED BCL2/IHG REARRANGEMENT MEASUREMENTS IN FOLLICULAR LYMPHOMA PATIENTS TREATED WITH FRONT-LINE AUTO-TRANSPLANTATION IN THE GELF-94 TRIAL BY THE GELA

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**Backgrounds.** This study was undertaken to assess the impact of molecular residual disease (MRD) on survival controlling for consolidation treatment of two regimens: an autotransplantation framework or chemotherapy with interferon, in the treatment of recurrent follicular lymphoma. MRD was defined as disappearance of Bcl2-IgH amplification by PCR. Aims: From 07/94 to 03/01, we have performed a prospective study, the GELF-94 trial, which randomized consolidation treatment after achieving clinical response between front line ASCT and chemotherapy. Of 401 patients included, 209 received 12 cycles of CHVP (cyclophosphamide, doxorubicin, vincristine and prednisone) then high-dose therapy with total body irradiation and ASCT (CHOP-HDT arm). Methods: Bone marrow (BM) and peripheral blood (PB) samples were obtained prospectively at diagnosis and repeated every 6 months during the first year then annually for PCR analysis in 12 laboratories. A standard PCR technique with one step of amplification was used for MBR and mcr breakpoints. Time repeated measurement was stopped at clinical relapse or instigation of a new treatment. At diagnosis, 225 patients had material available for Bcl2/Igh rearrangement analysis: BM for 182 (45%) patients and blood for 199 (50%). In the latter, 105 of the 199 patients (53%) were found to have a bcl2/IgH rearrangement in MBR breakpoint, whereas bcl2/Igh rearrangement in mcr was observed in 5 patients (3%) and no rearrangement at MBR or mcr in the remaining 94 patients (44%). No differences were found according to bcl2/Igh rearrangement in terms of response rate (82% for bcl2- vs. 80% for bcl2+) and 5 years survival (85% for bcl2- and 79% for bcl2+). Time repeated Bcl2/Igh rearrangement measurements were available for 142 patients (ASCT n=75, chemo n=67) in BM for 79 patients and in blood for 85 patients. There was no statistically significant difference in clinical characteristics between patients with/time without time repeated measurement. Results: At a median follow-up of 64 months, the significant prognostic factors for survival were age below 40 yr (HR= 21, p=0.005), complete clinical response (RR=5, p=0.02) and bcl2/Igh rearrangement negativity (RR=4, p=0.03), by time dependant Cox's model. There was no treatment impact. These findings confirm the importance of molecular response in addition to the clinical response as a critical factor for prognosis. Conclusion: No matter whether after chemotherapy alone of after autologous bone marrow transplant, patients in complete clinical and molecular remission show a significantly longer overall survival.

**1042**
GAINS ON CHROMOSOME BAND 18q21 PREDICT POOR OUTCOME IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA: RESULTS FROM A COMPARATIVE GENOMIC HYBRIDIZATION ANALYSIS WITHIN A MULTICENTRIC TRIAL (NHL-B-TRIAL)

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**Backgrounds.** In Non-Hodgkin lymphomas, there are only few data regarding the prognostic significance of specific genomic aberrations. No analyses correlating genomic aberrations with the clinical course have been performed in homogenous cohorts of patients treated within a clinical trial. Aims/Methods. We used comparative genomic hybridization (CGH) to perform such an analysis in diffuse large B-cell lymphomas (DLBCL). 367 paraffin-embedded tumor samples were obtained from patients, who where treated within the NHL-B trial of the German High-Grade Non-Hodgkin's Lymphoma Study Group. In this trial, all patients received similar therapy regimens (CHOP or CHOP-E) administered every 14 or 21 days. Results. CGH analysis was successful in 256 out of 567 cases (70%). 186 patients out of this series had a histology of DLBCL according to the WHO classification. In 137 of 186 cases (74%), unbalanced chromosomal aberrations were found (range from 1 to 25; median 4.5). The most frequent chromosomal changes (>15% of all cases) were gains involving chromosome bands 1q21 (16%), 7q11 (15%), 7q11 (16%), 17q22 (15%), 1q21 (16%), 17q22 (16%), 1q21 (16%), 5p15 (15%), 6q21 (25%) and 13q11 (25%). 43 high-level DNA amplifications were found in 26 cases, most frequently involving the chromosomal bands classified in two groups according to the presence (high tumor burden HTB) or not (low tumor burden LTB) of one of the following parameters: nodal or extranodal tumor mass with a diameter over 7 cm, involvement of three nodal sites (over 3 cm); B-symptoms; large splenomegaly; serous effusion; local risk of compression and leukemia or blood cytopenia. Patients with a HTB were randomly assigned to: no treatment until progression, prednimustine for 18 months or interferon α-2b for 18 months. Patients with a high HTB were randomly assigned to: CHVP (cyclophosphamide 600 mg/m², doxorubicin 25 mg/m², teniposide 60 mg/m² and prednisone 40 mg/m² × 5 days) monthly for 6 months then, after response, every two months for 12 months; CHVP plus interferon α-2b (5 MU/day 3 times per week) for 15 months.

**Initial prognostic factors were validated with 4.5 years longer median survival in patients with LTB but no plateau in both groups. For patients with LTB, the long term survival was similar according to the three randomization arms confirming that waiting for treatment until symptoms did not have any impact on prognosis. For patients with HTB, the benefit of adding interferon to a doxorubicin containing chemotherapy was confirmed (median survival 5.6 versus 7.6 years, p=0.01) With a 10 year follow-up 55% of patients are alive in the LTB group and 40% in the HTB, and 89 patients are still in first remission At first relapse 239 patients had documented progression and 85 histological transformation were gains involving chromosome bands 1q21 (16%), 7q11 (15%), 7q11 (16%), 17q22 (15%), 1q21 (16%), 17q22 (16%), 1q21 (16%), 5p15 (15%), 6q21 (25%) and 13q11 (25%). Conclusion, despite a long median survival in patients with FL, no plateau has yet been reached but some patients experienced a very long survival.

**Characteristics of patients**

<table>
<thead>
<tr>
<th>Low TB</th>
<th>High TB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age</td>
<td>52 y</td>
</tr>
<tr>
<td>Sex ratio M/F</td>
<td>1.2</td>
</tr>
<tr>
<td>Abnormal LDH</td>
<td>10%</td>
</tr>
<tr>
<td>Stage IV</td>
<td>60%</td>
</tr>
<tr>
<td>BM involvement</td>
<td>56</td>
</tr>
<tr>
<td>Median survival</td>
<td>11 y</td>
</tr>
</tbody>
</table>
findings suggest that MALT lymphoma frequently presents as a multi-
limited to cases with an additional 18q gain (p < 0.001). T(11;18)(q21;q21) was significantly more patients with extragastric MALT lymphoma had dissemination to the stomach. Out of these nine patients with sec-
t(14;18) involving IGH/MALT1 (p = 0.005). 15% of patients. We report our findings with an extensive staging
improve the risk assessment in DLBCL.

DNA 12p12 (p = 0.005) on chromosome arm 12p12 (p = 0.006, TTF) and on chromo-
4; 3q27: p < 0.001, OS, p = 0.002, TTF; 3q27: p < 0.001, OS, p = 0.001, TTF), on chromosome arm 18q21 (p < 0.001, OS, p = 0.002, TTF), as well as losses on 17p13 (p = 0.09, OS, p = 0.001, TTF) were associated with an inferior prognosis. In a multivariate model including the clinical parameters of the Interna-
trisomy 3 and 18 was significantly higher in patients with extragastric MALT lymphomas (12.5%, 8 in the colorectum and two
11 had MALT lymphoma originating in the lung (14%) and 10 had pri-
cluded in this context of a clinical lymphoma trial. Genomic changes can improve the risk assessment in DLBCL.

**1043 ASSESSMENT OF DISEASE DISSEMINATION IN GASTRIC VERSUS EXTRAGASTRIC MALT LYMPHOMA USING EXTENSIVE STAGING: A SINGLE CENTER EXPERIENCE OF 140 PATIENTS**


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**Backgrounds.** Molecular data as well as preliminary clinical findings have suggested MALT lymphoma as a multifocal disease in a high percentage of patients. We report our findings with an extensive staging routine applied in patients diagnosed with MALT lymphoma at our institution. **Patients and Methods.** A total of 140 consecutive patients underwent staging according to a standardized protocol. Sixty-one had gastric lymphoma, while 79 had been diagnosed with extragastric MALT lymphoma. The majority of these latter patients had salivary gland lymphoma (n=24, 30%, with 22 parotid and 2 submandibular gland lymphomas), 17 had lymphoma of the orbit/lacrimal gland (20%), another 11 had MALT lymphomas originating in the lung (14%) and 10 had primary intestinal MALT lymphoma (12.5%, 8 in the colorectum and two in the small intestine). The remaining patients suffered from lymphoma of the thyroid (n = 5), conjunctiva (n = 4), the breast (n=3), the liver (n=2) and the kidney (n=2). Staging included gastroscopy with multiple biopsies, endosonography of the upper gastrointestinal (GI) tract, computed tomography (CT) of thorax and abdomen, lymph node sonography, ophthalmologic assessment, MRI of salivary and lacrimal glands, and bone marrow biopsy. All lesions suggestive of lymphoma involvement were subjected to biopsy, if accessible, and biopsies were evaluated for MALT-lymphoma specific genetic aberrations by means of RT-PCR and/or FISH. These included association of t(11;18)(q21;q21), t(14;18)(q21;q21) involving IGH and MALT1, t(1;14)(p22;q21), t(3;14)(q14;q32) involving FOXP1 and IGH and trisomies 3 and 18. **Results.** Out of 140 patients, 52 (37%) were found to harbour multifocal MALT lymphoma involving multiple organs. In total, 15 of 61 patients with gastric lymphoma (25%) had multiorgan involvement. Eight out of these 15 patients showed synchronous spread to the GI tract (involvement of colon and/or rectum in 7 and small bowel in one patient), while 6 had disease at another non-GI site (for details see Table 1). Organs affected included the lung in three patients, and lung along with parotid, bladder plus spleen, kidney, lung plus bone marrow and bone marrow alone in one patient each. By contrast, significantly more patients with extragastric MALT lymphoma had dissemination to another MALT organ (37 of 79, 46%; p = 0.045). Nine of these 37 patients had dissemination to the stomach. Of these nine patients with secondary spread to the stomach, 4 had lymphoma originating in the lung, one each in the small intestine, the lacrimal gland, the parotid, the conjunctiva and the kidney, respectively. Only 3 of 140 (2%) patients had bone marrow involvement. t(11;18)(q21;q21) was significantly more common in gastric MALT lymphomas (p = 0.002). The rate of both trisomies 3 and 18 was significantly higher in patients with extragastric lymphoma (p = 0.003 for trisomy 3 and p = 0.007 for trisomy 18), as was t(14;18) involving IGH/MALT1 (p = 0.056). Multifocality was significantly associated with t(11;18)(q21;q21) in gastric lymphoma (p = 0.045) and with trisomy 18 in extragastric lymphomas (p = 0.011). **Conclusions.** Our findings suggest that MALT lymphoma frequently presents as a multifocal disease. Extragastric MALT-lymphomas are significantly more prone to dissemination than gastric MALT lymphomas.

**1044 ANTIBIOTIC THERAPY WITH DOXYCYCLINE IS AN ACTIVE TREATMENT AGAINST OCULAR ADNEXA MALT LYMPHOMA: FINAL RESULTS OF A MULTICENTER PROSPECTIVE PHASE II TRIAL**


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An association between ocular adnexal lymphoma of MALT-type (OAL) and Chlamydia psittaci (Cp) infection has been reported. Preliminary data suggest that patients (pts) with Cp-related OAL could achieve lymphoma regression after eradicating therapy with doxycycline, while data on the activity of this strategy in Cp-negative OAL are not available. In this multicentre prospective trial, 27 consecutive pts with OAL and measurable disease, at diagnosis (n=15) or relapse, were treated with doxycycline 100 mg, bid orally, for 3 weeks. Objective response was the primary endpoint. The presence of Cp DNA in lymphoma samples was evaluated by TETR-PCR. Tolerability was excellent in all pts but one. At a median follow-up of 18 months (range 3-45), response was complete (CR) in 6 pts and partial in 7 (ORR=48%; 95%CI:50%-66%); three pts had a response <50%, 9 had stable disease (3-15 mo.), two had progressive disease. Response was slow; 5 pts achieved the best response only after one year of follow-up (median time to the best response: 6 mo.). TETR-PCR resulted positive in 11 (41%) pts and negative in 16. Lymphoma regression was observed in both PCR-positive and -negative pts (64% vs. 58%; p=0.25), with a CR rate of 36% and 13% (p=0.18), respectively. Response rate was similar between pts with conjunctival and intra-orbital lymphomas (43% vs. 54%, p=0.71). The three pts with regional lymphadenopathies and three of the 5 pts with bilateral OAL achieved objective response (4 CRs), which lasted 3+, 13+, 16+, 22+, 26+, and 37 mo. In relapsed pts, objective response was observed in 3 of 5 previously irradiated pts and in 5 of 7 non-irradiated pts (p=0.99). Twenty pts are failure-free, with a 2-yr FFS of 66±12%. Doxycycline is a fast, cheap and safe therapy, able to induce durable regression in 64% of Cp-related OAL. This antibiotic is a valid alternative against OAL, even in pts with multiple failures, involving previously irradiated areas or regional lymph nodes. We report for the first time responses also in PCR-negative OAL, this finding stimulates the development of more sensitive and specific methods for Cp detection and the study of potential associations with other infectious agents responsive to doxycycline.
The association between gastrointestinal angiodysplasia and von Willebrand’s disease (vWD) is uncommon. Since the first description in 1967, some other 20 cases have been reported; most of them were vWD type 2 and 3. The efficacy of several therapies has been inconsistent and transient: transfusions, factor VIII / vWD concentrates, endoscopic sclerosis, estrogens, surgery. Bowers et al. published 2 cases with this association in whom a significant decrease in transfusion requirements and episodes of hemorrhage was obtained through the use of octreotide (Br J Haematol. 2000 Mar;108(3):524-7). The therapeutic effect of this synthetic analogue of somatostatin in this setting lies on the reduction of splanchnic blood flow to abnormal blood vessels. In one of their patients, an unforeseen increase in vW factor activity was also demonstrated. We describe an additional case with protracted use of octreotide in a long-acting formulation. 61 yr. old, female, allergic to iodine (anaphylaxia), vWD type 2b. Chronic hepatitis C of presumed post-transfusion origin, genotype 1. Multifocal angiodysplasia in gastrointestinal tract with recurrent episodes of upper and lower bleeding (>100 days of hospital stay and >100 blood products per year). Previous therapies: surgery (partial gastrectomy, hemicolectomy); combined estrogens and progesterone.

To our knowledge, we report the first case of a patient with type 2B-vWD with multifocal angiodyplasia and multiple episodes of gastrointestinal bleeding. While octreotide-LAR is an option to be considered in those patients with recurrent bleeding related to vWD and gastrointestinal angiodysplasia, the long-term use of octreotide-LAR in a patient with type 2B-von Willebrand disease, multifocal angiodyplasia and multiple episodes of gastrointestinal bleeding requires further investigation.

**Table 1. Evolution of parameter.**

<table>
<thead>
<tr>
<th>Years after start</th>
<th>Blood products to hospital</th>
<th>Admittances of stay</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1999</td>
<td>137</td>
<td>18</td>
<td>123</td>
</tr>
<tr>
<td>2000</td>
<td>168</td>
<td>22</td>
<td>167</td>
</tr>
<tr>
<td>2001</td>
<td>87</td>
<td>16</td>
<td>161</td>
</tr>
<tr>
<td>2002</td>
<td>131</td>
<td>16</td>
<td>122</td>
</tr>
<tr>
<td>2003</td>
<td>80</td>
<td>13</td>
<td>96</td>
</tr>
<tr>
<td>2004</td>
<td>77</td>
<td>9</td>
<td>90</td>
</tr>
</tbody>
</table>

In the second semester of 2000, we obtained permission for compassionate use of octreotide; the initial therapeutic scheme included a progressively higher dose to reach 250 µg SC t.i.d., even though compliance to side effects prevented a dose higher than 250 µg SC b.i.d. At the end of 2000, the conventional formulation was replaced by long-acting-release (LAR) octreotide, 20 mg IM monthly, each dose proceeded by 1000 IU of a factor VIII / vWD concentrate to minimize local bleeding; both octreotide and factor were administered in the Day-In Hospital. The favourable evolution since then, with regard to decrease in number of hospital admittances, days of hospital stay and number of blood products transfused, is depicted on Table 1. In 2002, there was an apparent loss of response, related to a single episode of hemopteritoneum. In 2004, two of the admittances were due to thrombosis and infection of the central venous catheter, which required heparin therapy. When comparing data from 1999 and 2000 on one side, and those from the following 4 years on the other, the number of transfused blood products, hospital admittances as well as days of hospital stay, diminished by a gross one third (39%, 35% and 30%, respectively), with a trend to further decrease. In addition, this has led to a significant improvement in the quality of life for the patient, allowing her a ‘life beyond the Hospital’.

The toxicity has been scarce, with a slight increase in blood pressure and glucose levels as the only relevant events. We believe that octreotide-LAR is an option to be considered in those patients with recurrent bleeding related to vWD and gastrointestinal angiodysplasia.

**1045**

LONG-TERM USE OF OCTREOTIDE-LAR IN A PATIENT WITH TYPE 2B-VON WILLEBRANDS DISEASE, MULTIFOCAL ANGIODYSPLASIA AND MULTIPLE EPISODES OF GASTROINTESTINAL BLEEDING

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The association between gastrointestinal angiodysplasia and von Willebrand’s disease (vWD) is uncommon. Since the first description in 1967, some other 20 cases have been reported; most of them were vWD type 2 and 3. The efficacy of several therapies has been inconsistent and transient: transfusions, factor VIII / vWD concentrates, endoscopic sclerosis, estrogens, surgery. Bowers et al. published 2 cases with this association in whom a significant decrease in transfusion requirements and episodes of hemorrhage was obtained through the use of octreotide (Br J Haematol. 2000 Mar;108(3):524-7). The therapeutic effect of this synthetic analogue of somatostatin in this setting lies on the reduction of splanchnic blood flow to abnormal blood vessels. In one of their patients, an unforeseen increase in vW factor activity was also demonstrated. We describe an additional case with protracted use of octreotide in a long-acting formulation. 61 yr. old, female, allergic to iodine (anaphylaxia), vWD type 2b. Chronic hepatitis C of presumed post-transfusion origin, genotype 1. Multifocal angiodysplasia in gastrointestinal tract with recurrent episodes of upper and lower bleeding (>100 days of hospital stay and >100 blood products per year). Previous therapies: surgery (partial gastrectomy, hemicolectomy); combined estrogens and progesterone. She refused prophylactic use of vW / factor VIII derivatives.

In the second semester of 2000, we obtained permission for compassionate use of octreotide; the initial therapeutic scheme included a progressively higher dose to reach 250 µg SC t.i.d., even though compliance to side effects prevented a dose higher than 250 µg SC b.i.d. At the end of 2000, the conventional formulation was replaced by long-acting-release (LAR) octreotide, 20 mg IM monthly, each dose proceeded by 1000 IU of a factor VIII / vWD concentrate to minimize local bleeding; both octreotide and factor were administered in the Day-In Hospital. The favourable evolution since then, with regard to decrease in number of hospital admittances, days of hospital stay and number of blood products transfused, is depicted on Table 1. In 2002, there was an apparent loss of response, related to a single episode of hemopteritoneum. In 2004, two of the admittances were due to thrombosis and infection of the central venous catheter, which required heparin therapy. When comparing data from 1999 and 2000 on one side, and those from the following 4 years on the other, the number of transfused blood products, hospital admittances as well as days of hospital stay, diminished by a gross one third (39%, 35% and 30%, respectively), with a trend to further decrease. In addition, this has led to a significant improvement in the quality of life for the patient, allowing her a ‘life beyond the Hospital’.

The toxicity has been scarce, with a slight increase in blood pressure and glucose levels as the only relevant events. We believe that octreotide-LAR is an option to be considered in those patients with recurrent bleeding related to vWD and gastrointestinal angiodysplasia.
DYSKERATOSIS CONGENITA: UNUSUAL PRESENTATION WITH TROMBOCYTOPENIA IN EARLY AGE

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Backgrounds. Dykeratosis congenita (DC) is rare, usually fatal inherited skin and bone marrow (BM) failure syndrome that displays considerable genetic and clinical heterogeneity. Classical DC is an inherited BM failure syndrome characterized by the mucocutaneous trials of abnormal skin pigmentation, nail dystrophy and mucosal leucoplaclia A variety of the other somatic abnormalities have also been reported. At genetic level X-linked recessive, autosomal dominant (AD) and autosomal recessive (AR) forms of the disease exist where the genetic basis of the X-linked and AD forms have been determined. The X-linked form of DC is due to mutations in DCK1, the gene encodes dyscerin, a protein that is part of telomerase complex. Autosomal dominant DC is caused by mutations in TERC, which codes for the RNA component of telomerase. The DCK1 gene is expressed in all tissues of the body consistent with it having a housekeeping function in the human cell. This correlates well with the multi system phenotype of DC. Aims. In this report we present unusual onset of DC with trombocytopenia as a first presentation at an early age. Methods/Results. A 4 year old male patient with DC, presented at the age of 18 months with isolated trombocytopenia, associated characteristic skin finding and nail dystrophy. The trombocytes count of 24,000/mm² persisted without the presence of anemia or Neutrogera. Trombocytopenia responded to kortikosteroids at a dose of 2.5 mg/kg per day. Six months late he was admitted in the hospital with severe anemia; trombocytopenia and neutrogera. Severe aplastic anemia was later diagnosed. Clinical examination showed hyperpigmentation over the neck, and nails dystrophy. All the nails were dystrophic. To substantial the diagnosis the genes responsible for the X-linked and AD forms of DC (DCK1 and TERC) were screened for mutations. No abnormal patterns have been detected in patient in either gene. However, his mother appears to have a highly skewed pattern of X-chromosome inactivation which is characteristic observed in carriers of DCK1 mutations. Conclusions. This patient’s clinical course is interesting because the thrombocytopenia developed as an isolated symptom at the age of 18 months and preceded the skin abnormalities. The diagnosis of dykeratosis congenita was made only after an evolution. The diagnosis of dykeratosis congenita, should be considered in all patients with isolated trombocytopenia, along with platelet defects. Here we report the successful use of platelet transfusion along with platelet suspensions which was followed by 50 microg/kg repeated doses at 2-h intervals for two times. The bleeding was taken to control and the symptoms of the patient improved gradually and she was discharged without any sequel. Patient 2 was an 18 year old girl with therapy resistant Fanconi’s Anemia in whom refractoriness was developed. She suffered from severe life-threatening anemia. In this patient refractoriness could not be controlled by repeated thrombocyte transfusions and oral contraceptive therapy. Recombinant FVIIa at initial dose of 90 microg/kg was given and it was repeated at the same doses at 24 hours intervals for two times. Her hemorhage decreased gradually and ceased completely after the third injection of rFVIIa. No side effects related to use of rFVIIa has been observed in these two patients. We concluded that recombinant factor VIIa should be considered as a therapy choice in life-threatening bleeding of the patients with therapy resistant immune thrombocytopenia and aplastic anemia in whom thromboocyte refractoriness is developed.
for all investigations. This analyser enables quantitative measurement of platelet-mediated haemostasis in noncoagulable (citrated) blood. The method simulates platelet activation by mechanical stress - shear stress, and also simulates contact of platelets with collagen. There were 9 patients with familiar hypercholesterolemia in the study group (4 females and 5 males). Age ranges from 17 to 59 years (46.4 years average age and 38 years median). 2 of them have homozygous hypercholesterolemia. Our aim was to investigate the changes before and after procedures two times in every patient. Results. 18 pairs of samples were examined using COL/EPI membrane (collagen/epinephrine) and 17 pairs of samples were examined using COL/ADP membrane (collagen/ADP), total number of samples was 70. Closure time (CT) values were prolonged after separation in all cases but CT prolongation was not statistically significant (p>0.14). No differences between homozygous and heterozygous patients were found. Summary/Conclusions. Investigation of primary haemostasis immediately after procedures using PFA-100 analyser is not a suitable marker and could not be used to determine the optimal intensity of particular LDL-apheresis procedures.

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TWINSING IS A SIGNIFICANT INDEPENDENT FACTOR IN THE DEVELOPMENT OF CHILDHOOD MALIGNANCIES
M. Moser, Y.B. Bodenheim, A.C. Cohen, I.H.V. Har-Vardi, J.K. Kapelushnik

Soroka Medical Center, Beer Sheva, Israel; Clalit Health Services, Beer Sheva, Israel; Reproduction Unit, Soroka Medical Center, Beer Sheva, Israel

In the past few years we have noticed a marked increase in the incidence of cancer among twins treated at the Pediatric Hematology-Oncology Unit (PHOU) of Soroka Medical Center (SMC) in Beer Sheva, Israel. In this work, we reviewed the relationship between twinning and the risk of developing cancer during early childhood. Children born at SMC between Jan. 1991 and Sep. 2008, with any malignancy were included in the study if they were under 13-years-of-age at time of diagnosis. Three controls of the same sex were matched to each patient from the birth registries of the same day at SMC. A twin was not chosen as a control to its sibling. Data from patients, controls, and mothers were collected from medical records and included three areas of investigation: demographics and obstetric history of the mothers, delivery data, and gestational events and/or interventions (infertility including in vitro fertilization (IVF), ART, diabetes mellitus (DM), hypertension (HTN), urinary tract infections (UTI), iron deficiency anemia (IDA), and medications). A total of 145,087 deliveries, resulting in 145,503 children, were registered at the Soroka Medical Center (SMC) between January 1991 and September 2008. Of those, 98.37% were singleton and 1.63% were multiple births (2,261 twins; 77 triplet and quadruplets), cumulating in 972,000 patient years of follow up. The crude incidence of cancer during childhood is 14,100,000 per year, while the incidence of cancer calculated for the children born during the study period was 10.5:100,000 per year. Of the 92 children with cancer, complete information was obtained for 65 patients (70.6%); eight (12.3%) were twins, four were born after ART (6.7%), two of whom were twins. Significantly more Bedouin children were found in the patients group. According to this data, the total expected number of cancer cases among twins born during the study period was 2.23, while the total observed number at the PHOU is 3.59 times higher (8 cancer cases), p<0.001. Twinning per se was found to be an independent factor in the development of childhood cancer. Although seeming significant in a univariate analysis, this increase can be a marker of total body iron amount may be an indicator of total iron accumulation. Therefore non invasive, salive samples for measurement of iron and ferritin may prefer instead of blood samples in patients with thalassemia. For this reason, we think that more extensive and controlled studies are needed to use the saliva as a routine diagnostic material.

1054
FERTILITY AND REPRODUCTION IN THALASSEMIA MAJOR
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1 Polichinico ‘G. Martino’ Medical School, Messina, Italy; 2 Department of Paediatric Sciences, Messina, Italy; 3 Department of Neurosciences, Psychiatric, Messina, Italy

Backgrounds. Therapeutic advances in thalassemia major have increased the average lifespan and improved the quality of life patients. Attainment of reproductive capacity and creation of a family has become a challenging task. Hypogonadotropic hypogonadism due to hypothyroidism is still present and become a barrier in their desire for parenthood. Nowadays women with thalassemia can safely complete pregnancy, but the decision to conceive has to be carefully considered by a couple in consultation with their doctors. Patients with thalassemia who have a normal menstrual cycle may conceive spontaneously. Those suffering from primary or secondary amenorrhea can be submitted with hormonal treatment in order to stimulate the production of ova and the induction of ovulation. Aims. Aim of our study is to estimate the frequency of fertility (spontaneous or after induced ovulation) and pregnancy complications for mother and newborn, in patients admitted to the Paediatric Department - Thalassaemia Ward G. Martino Polichinico. Patients and Methods. We followed 36 women mean age 32 (18-46) years. All patients were treated according to the standard treatment protocol. 9/36 women with Thalassemia Major became pregnant and were the object of our study. At the beginning of pregnancy, average age was 26 years. Five pregnancies were spontaneous and four were induced after ovarian stimulation followed by natural insemination. Women who expressed the desire to become pregnant underwent a complete evaluation of psychological and clinical conditions. Glucose tolerance, thyroid, serum ferritin levels, hepatic and renal function tests, bidimensional echocardiography were performed before, during the pregnancy and after delivery. Also Bone Mineral Density (BMD) was measured, by the DEXA method, before pregnancy and after delivery. Once the patients were confirmed to be pregnant, iron chelator treatment was stopped. Mean pre-transfusional Hb and blood consumption have been monitored in order to keep pre-transfusional Hb at 10-10,5 g/dl levels. A complete obstetrical survey was performed every two weeks. Results. Our findings show that 8 babies were delivered by elective caesarean section at 37+ weeks of gestational age (GA). The mean birthweight of the newborns was 2954 g. All babies were normal. Ferritin levels increased during pregnancy in all excesser hemolysis, increased intestinal iron absorption and frequently blood transfusions that causes organ damage and dysfunction. Especially in childhood period, serum iron level measurement methods are technically invasive. Difficulty of the current methods used to evaluate the iron accumulation in organs suggests the importance of saliva usage for diagnosis. In this study, it has been supposed that salivary iron amount could be a marker of total body iron amount may be an indicator of total iron accumulation. Therefore non invasive, salive samples for measurement of iron and ferritin may prefer instead of blood samples in patients with thalassemia. Material and Methods. 34 healthy children as control group were compared with 71 thalassemia major, 10 thalassemia intermedia and 15 thalassemia trait. Salivary and serum iron and ferritin levels were measured in all groups. Results. there was no statistically significant difference between the control group and other groups by means of age and gender (p>0.05). There were no correlation between serum and salivary iron and ferritin levels in thalassemia major, intermedia and trait groups (Table)

Table. Correlation between serum and salivary iron and ferritin levels in patients thalassemia.

<table>
<thead>
<tr>
<th>Control</th>
<th>Salivary and Serum Iron</th>
<th>Salivary and Serum Ferritin</th>
</tr>
</thead>
<tbody>
<tr>
<td>r = 0.885, p = 0.000**</td>
<td>r = 0.842, p = 0.000**</td>
<td></td>
</tr>
<tr>
<td>T. Major</td>
<td>r = 0.972, p = 0.000*</td>
<td>r = 0.364, p = 0.034*</td>
</tr>
<tr>
<td>T. Intermedia</td>
<td>r = 0.720, p = 0.019**</td>
<td>r = 0.891, p = 0.001**</td>
</tr>
<tr>
<td>T. Trait</td>
<td>r = 0.955, p = 0.000**</td>
<td>r = 0.831, p = 0.000**</td>
</tr>
</tbody>
</table>

As a conclusion, salivary iron and ferritin levels increase as well as serum. This increase may be a marker of total body iron amount may be an indicator of total iron accumulation. Therefore non invasive, salive samples for measurement of iron and ferritin may prefer instead of blood samples in patients with thalassemia. For this reason, we think that more extensive and controlled studies are needed to use the saliva as a routine diagnostic material.
patients. After delivery all of them were in good general conditions and were treated with intensive iron chelation in order to reduce iron overload. No changes in laboratory parameters, BMD, ecocardiography evaluation hepatic and renal functions, have been observed besides increased iron stores. There were no delivery complications but one case of intrauterine death at the 35th weeks due to acute placental injury and one abortion in the early pregnancy were reported. Conclusions. Pregnancy can be safe in mothers and babies if closely monitored. Reproduction in patients with thalassaemia major is becoming a new reality, allowing them an improved quality of life. Maternity desire has to be considered with special caution and sensitivity. An optimal relationship has to be reached between patients and care-givers to improve patients’ safety. This demand effort and embraces all disciplines and sectors requiring a comprehensive, multifaceted approach to identify and manage actual and potential risks.

Table 1. Mean differences and T-test p-values.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Mean improvement</th>
<th>Hb (g/dL)</th>
<th>FERR (µg/dL)</th>
<th>MCV (FL)</th>
<th>MCH (pg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALL patients</td>
<td>2.0 (p&lt;0.001)</td>
<td>113.5 (p&lt;0.001)</td>
<td>6.9 (p&lt;0.001)</td>
<td>2.8 (p&lt;0.001)</td>
<td></td>
</tr>
<tr>
<td>ACD</td>
<td>1.12 (p=0.047)</td>
<td>128.8 (p=0.011)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CKD</td>
<td>2.58 (p=0.008)</td>
<td>154.8 (p=0.008)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GAST</td>
<td>2.01 (p&lt;0.001)</td>
<td>69.5 (p&lt;0.001)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Menor</td>
<td>3.0 (p=0.001)</td>
<td>118.3 (p=0.022)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Patients were anaemic due to either anaemia of chronic disease (ACD) (F=9; M=0), chronic kidney disease (CKD) (F=3; M=3), gastrointestinal related diagnosis (GAST) (F=22; M=9) or menorrhagia (MENOR) (N=7).

1056 POST-TRAUMATIC STRESS DISORDER IN CHILDREN AFFECTED BY SICKLE CELL DISEASE AND THEIR PARENTS

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Backgrounds. Children affected by SCD suffer from recurrent painful crises, some of them being life threatening, or felt as life threatening by children and/or their parents. Aim. We hypothesized that painful crises could generate PTSD in some children, PTSD being described in patients having experienced or witnessed an event that involved an actual or threatened injury to physical integrity of self or others. Patients with PTSD present symptoms in each of these categories: reexperiencing, avoidance/numbing, increased arousal. Until now, PTSD had never been described in SCD children. Methods. We enrolled 11 SCD children, 9 males, 2 females hospitalized at least once for a painful crisis, at least one month before the study, who accepted with one of their parents to participate in the study. Their mean age was 11.6±2.2 years (range: 7.5-15.5). One father and 10 mothers were also studied, one mother being secondarily excluded from analysis because she did not answer the questions. Both children and parents had to answer to a semi-structured interview (SCID) and to complete questionnaires (IES-R, STAY-Y, BDI-II, CDI, CBCL). Socio-demographic data and medical past histories were recorded. Statistical analysis used exact Fischer test and Mann Whitney test. Results. Three children had a PTSD (27%), and 4 parents (40%). We found no correlation between PTSD and socio-demographic data. Mean numbers of hospitalization/year were respectively 1.1±0.5 and 1.30±0.5, and mean number hospitalization in intensive care units reached 0.5±0.6 in children with and without PTSD (N.S.). PTSD presence was not correlated in children and parents. There was a correlation between PTSD and the parents’ feeling of powerlessness on their child’s illness (p=0.04). Summary/Conclusions. Painful crises are the most frequent complications of SCD, and one of the most difficult question for their management is the assessment of pain intensity. We show here that PTSD could be a complication of SCD. Symptoms such as intrusive distressing recollections of past painful events may worsen the consequences of vasocclusion, enhancing children’s feeling of pain, and leading physicians to an inadequate use of analgesics instead of psychological support. Furthermore, stress is a precipitating factor for vasocclusive crises and may facilitate recurrences of painful episodes. Moreover, PTSD itself causes psychological distress and may disturb children’s development. Interestingly, PTSD was not related in our study to the objective severity of the disease. Looking for PTSD and proposing specific individual and family psychological interventions could very probably contribute to disrupt the vicious circle between pain and fear of pain in SCD children.
packed red blood cells; 7 and 14 mL/kg/month correspond with approximately 2 and 4 adult units of blood, respectively. Results. In the overall population, 146 (22.4%), 419 (64.3%) and 87 (13.3%) patients who received deferasirox while on study had low, intermediate and high transfusional requirements, respectively; the equivalent numbers for patients receiving DFO were 61 (17.3%), 255 (66.6%) and 57 (16.1%), respectively. In completing patients with an LIC assessment at baseline and study end (approximately 90% of the overall population), a transfusion- and dose-related pattern was observed in the response of LIC (Figure 1) and serum ferritin. This was observed for both deferasirox (n=566) and DFO (n=327). Changes in iron burden were similar at comparable therapeutic doses. Conclusions. Transfusional requirement has a clear impact on response to chelation therapy. Physicians are able to tailor deferasirox dose according to patient needs, with dosing based on transfusion rate, severity of iron overload and treatment goal. Using this method, deferasirox was shown to meet the individual requirements of an extremely high proportion of patients treated. Deferasirox 10 mg/kg/day maintained iron balance in patients with low transfusional requirements, 20 mg/kg/day maintained or reduced iron balance in patients with low and intermediate requirements, while 30 mg/kg/day reduced iron balance in patients with high transfusional requirements. As regular transfusions lead to rapid iron accumulation, it is essential to monitor patients for the number of blood units transfused, serum ferritin levels and/or LIC. Across a range of transfusion-dependent anemia care and transfusional requirements, DFO and deferasirox in a 2:1 dose ratio have comparable effects on LIC and serum ferritin.

Figure 1. Change in LIC (mg Fe/g dw), by treatment, dose and transfusion-patient numbers in these cohorts.

*Data not shown for the deferasirox 5 and DFO <25 mg/kg/day dose cohorts due to low patient numbers in these cohorts.

**1058**

IRON OVERLOAD CAUSED BY REPEATED TRANSFUSIONS IN ADULT CHRONIC REFRACTORY ANEMIAS

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Many patients with refractory anemia suffer from iron overload and associated complications. However, it is not yet established when to start deferoxamine therapy to prevent this. To analyze the clinical features of iron overload caused by repeated transfusions in adult chronic refractory anemias. We chose patients who received more than ten units of RBC cells from the database of blood bank in our institute and their medical records were retrospectively reviewed. Twenty-nine patients (M: 18, F: 11) were identified and median age was 52 years (range: 22-82). Underlying disease causing repeated transfusions was aplastic anemia in 11 patients, myelodysplastic syndrome in 2 and multiple myeloma in 2 patients. Each of the remaining patients had pure red cell aplasia, chronic lymphocyte leukemia, non-Hodgkin’s lymphoma, chronic myelogenous leukemia and acute myelogenous leukemia. Patients received median 85 units of RBC transfusions (range: 26-226). Two patients received 45, 101 units of RBC transfusions and developed liver cirrhosis after 143, 184 months from the initial diagnosis of their underlying diseases, respectively. Cardiomyopathy developed in 4 patients after 29, 100, 104, and 143 months from the diagnosis of underlying diseases. They received 71, 105, 115 and 135 units of RBC, respectively. Diabetes mellitus developed in 5 patients. Twenty-four patients started deferoxamine therapy when they received median 48 units of RBC (range: 18-164). Eight patients already had complications (liver cirrhosis: 2, cardiomyopathy: 2, diabetes mellitus: 4) at the time of starting deferoxamine. Eleven patients received treatment for underlying disease on a curative intent (allogeneic stem cell transplantation, 7; autologous transplantation, 1; combination chemotherapy, 3), but only three of them responded to deferoxamine and three patients died of complications associated with treatment for underlying disease. Two patients died of cardiomyopathy. Median overall survival after the diagnosis of underlying disease was 150 months. Iron overload is a common complication of adult chronic refractory anemia. The risk of developing serious complications increased with the increase of RBC transfusions. Patients who developed cardiomyopathy had a worse prognosis especially and therapy should be started earlier to prevent associated complications.

**1059**

FERRITIN LEVELS, NON-COMPLIANCE AND ADVERSE EVENTS IN RELATION TO INFUSED IRON CHELATION THERAPY IN AN INTERNATIONAL COHORT OF PATIENTS FROM ACTUAL PRACTICE

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Background. Deferoxamine (DFO) is an iron chelation therapy (ICT) administered to patients undergoing chronic blood transfusions. Although efficacious, it is burdensome to patients because of the necessity of almost daily infusions lasting 8 to 10 hours each (2000–2700 mg iron/dose) to prevent occurrence of treatment related adverse events. Non-adherence to ICT, however, results in iron overload which, if not removed, results in serious clinical and economic outcomes such as myocardial, endocrine and hepatic dysfunction. Aims: To document ferritin levels, non-compliance and prevalence of adverse events in a cohort of patients undergoing infused ICT. Methods: A retrospective, semi-prospective study of the economic and quality of life burden of infused ICT in the usual care setting was undertaken. Compliance and adverse events were obtained from patient interviews. Serum ferritin level data and adverse events experienced by these same patients during their initial and most recent year of ICT therapy were collected from the patients’ medical charts. Results. 78 patients (44% male; mean age: 28.9 ± 14.6 years) with thalassemia (n=51), sickle cell disease (n=23), and myelodysplastic syndrome (n=4) were recruited from 8 different sites within the US (4 sites) and the UK (4 sites). Sixty per cent of patients reported non-compliance to ICT over the previous 2 years. Of these, 49% could be considered at risk for iron overload complications because they reported missing more than 2 out of 5 doses. Over the previous 30 days, 64% of patients suffered at least one adverse event; those most commonly reported were site soreness (86%), site irritation, (74%), ringing in the ears (22%) and abdominal pain (20%). Of the 56 (75%) patients who had missed at least one dose during the past 4 weeks, 23% did so because of adverse events to ICT. During the initial year of ICT, the adverse events documented in the charts of 8 patients were injection site soreness/rash (30%), allergic reaction to medication (13%), breathing problems (13%) and nausea (13%) while in the most recent year of ICT, the adverse events most commonly reported by 15 patients were site soreness/rash (31%), tinnitus (13%) and joint pain (13%). Serum ferritin level test results obtained from charts indicate that, in general, average blood iron levels are somewhat high and increase slightly over time despite ICT. In some patient categories, this increase is more pronounced. For the initial year of ICT, the mean serum ferritin level was 2,618±1,577 ng/mL (US:2,519±1,382, UK:3,013±1,570) and 2,766±2,272 ng/mL (US:2,741±2,532, UK:2,813±1,740) for the most recent year. For patients for whom data of the most recent year and the first year of ICT were available, the mean serum ferritin level increased by 394.1± 2,633 ng/mL (US:2,741±2,532, UK:1,029±1,225) over that time period. For patients for whom data of the most recent year and the first year of ICT were available, the mean serum ferritin level increased by 394.1± 2,633 ng/mL (US:2,741±2,532, UK:1,029±1,225) over that time period. For patients for whom data of the most recent year and the first year of ICT were available, the mean serum ferritin level increased by 394.1± 2,633 ng/mL (US:2,741±2,532, UK:1,029±1,225) over that time period. For patients for whom data of the most recent year and the first year of ICT were available, the mean serum ferritin level increased by 394.1± 2,633 ng/mL (US:2,741±2,532, UK:1,029±1,225) over that time period. For patients for whom data of the most recent year and the first year of ICT were available, the mean serum ferritin level increased by 394.1± 2,633 ng/mL (US:2,741±2,532, UK:1,029±1,225) over that time period. For patients for whom data of the most recent year and the first year of ICT were available, the mean serum ferritin level increased by 394.1± 2,633 ng/mL (US:2,741±2,532, UK:1,029±1,225) over that time period. For patients for whom data of the most recent year and the first year of ICT were available, the mean serum ferritin level increased by 394.1± 2,633 ng/mL (US:2,741±2,532, UK:1,029±1,225) over that time period. For patients for whom data of the most recent year and the first year of ICT were available, the mean serum ferritin level increased by 394.1± 2,633 ng/mL (US:2,741±2,532, UK:1,029±1,225) over that time period.
RESPONSE RATE IN IDIOPATHIC THROMBOCYTOPENIC PURPURA. SINGLE INSTITUTION EXPERIENCE

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Backgrounds. Idiopathic thrombocytopenic purpura (ITP), also referred as immune or autoimmune thrombocytopenia, is an acquired disease characterized by low platelet count, normal bone marrow, usually with an increased number of megakaryocytes and the absence of any other disease. The majority of patients respond, on short term, to an initial corticosteroid therapy, but this approach does not influence the natural evolution of the disease on long term, as only about a third of the patients remain in sustained remission at the cessation of treatment. Aims. The study analysed retrospectively the therapeutic response in patients with ITP followed in our institution between 1996 and 2005. Method. A precise number of test was done to all thrombocytopenic patients, at the time of differential diagnosis a positive ITP diagnosis was established in 43 patients, 34 women and 9 men. They were treated commonly with various doses of corticosteroids. Other administered treatments included intravenous immunoglobulin (IVIG), splenectomy, vinca alkaloids, platelet concentrate and rituximab. We consider a sustained response a platelet count above 50,000/µL or above 30,000/µL without hemorrhages or only with minor purpura. A complete response was considered a platelet count above 150,000/µL without hemorrhages or only with minor purpura. A complete response was obtained in 25 cases (62.5%), was partial in 5 cases and in 10 patients there were no response. The complete response to corticosteroids was sustained only in 6 cases (15%). In other 11 cases the response was sustained but the platelet count was well below 150,000 (total response rate 39.53%). In 10 cases (65%) a dose of prednisone was decreased and in 4 cases the dose of prednisone needed decreased and in 2 cases no effect was observed. There was only one death (15 years after diagnosis) in our study (2.32%) and no severe infection in patients with splenectomy. Conclusions. The diagnosis of ITP covers a large spectrum of patients, with low mortality, but with important morbidity and treatment difficulties. Response to corticosteroids was as predicted. Splenectomy was consider after 3 to 6 months in patients resistant to corticosteroids or earlier at patient demand. The response to corticosteroids, appreciated by the raise of platelets count, in the 40 patients treated this way, was obtained in 25 cases (62.5%), was partial in 5 cases and in 10 patients there were no response. The complete response to corticosteroids was sustained only in 6 cases (15%). In other 11 cases the response was sustained but the platelet count was well below 150,000 (total response rate 39.53%). In 10 cases (65%) a dose of prednisone was decreased and in 4 cases the dose of prednisone needed decreased and in 2 cases no effect was observed. There was only one death (15 years after diagnosis) in our study (2.32%) and no severe infection in patients with splenectomy. Conclusions. The diagnosis of ITP covers a large spectrum of patients, with low mortality, but with important morbidity and treatment difficulties. Response to corticosteroids was as predicted. Splenectomy was curative in only 40% of patients previously resistant to corticosteroids. A complete response to corticosteroids was observed repeatedly in our single fatal case.
child did not finish the treatment due to allergic reaction. Two other children did not respond. Three patients with AIA has been in CR for 1 to 2.4 years and ongoing. One patient (21 years-old) had CR for only one month. The patient with Evans syndrome has been in CR for 14 months and ongoing. Therapy was well tolerated, except for an allergic reaction in two patients, and no infectious complications occurred. Steroids were withdrawn in patients in CR. The CD20+ B cell count decreased in most patients to less than 1% after Rituximab. Patients with refractory chronic immune cytopenias respond well to Rituximab, even after splenectomy. Rituximab may be considered before splenectomy in patients with high risk of complication, and it allows to withdraw steroids when the patient enter in CR.

1063 QUANTIFICATION OF SEMINAL THROMBOMODULIN
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Backgrounds. Semen forms a gel-like-coagulum immediately after ejaculation, embracing the sperm. Subsequently semen liquefies spontaneously after the presence of fibrin degradation products, prothrombin fragments, and other active components of the plasma clotting system in seminal plasma have previously been reported. Aim: To investigate the presence of thrombomodulin in human semen. Materials and Methods. Using an Imubind® Thrombomodulin ELISA assay - seminal thrombomodulin antigen levels were measured in 57 semen specimens obtained from sub-fertile, normally fertile, fertile sperm donors and vasectomized subjects. Results. Thrombomodulin was quantifiable in human semen. The vasectomy group showed the lowest value. Slightly higher levels were seen for the normal, fertile sperm donors and the pooled normal semen parameters stratification group (derived from the World Health Organization [WHO] fertility criteria) compared to the infertile subjects. However, there were no significant differences between these groups when tested against each other. Seminal thrombomodulin levels showed negative association with total sperm concentration (density), sperm counts per ml, days of abstinence, liquefaction time and semen volume. Conclusion: Our results establish the presence of thrombomodulin in human semen. Thus, provide further evidence for some involvement of the conventional haemostatic system in the coagulation and liquefaction properties of human semen.

1064 CLONAL PATTERNS OF HEMATOPOIETIC STEM CELLS IN PERIPHERAL BLOOD OF PERSONS ACCIDENTALLY EXPOSED TO HIGH DOSES OF RADIATION
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Background. The polyclonality of hematopoiesis was revealed using individually marked hematopoietic stem cells (HSC) in mice, dogs and primates. The insertion site analysis of human hematopoietic cells engrafted in immune-deficient mice identified several individual clones that contributed to hematopoiesis. However, the relevance of these xenograft models to natural human hematopoiesis in vivo remains unclear. HSC of persons exposed to high doses of radiation bear stable chromosome aberrations during whole life. Aims. The dynamics of HSC functioning in vivo is poorly understood because of the difficulty in clonally tracking individual stem cells. The goal of this study was to investigate clonal contribution to hematopoiesis in humans. Methods. Stable chromosome aberrations in individual HSC were used to evaluate the fate of distinct human hematopoietic clones bearing unique chromosome markers in peripheral blood cells in persons after high-dose irradiation and consecutive hematological recovery. Clonal chromosome aberrations were evaluated in individual colonies in semisolid medium developed from peripheral blood cells and in PHA-stimulated lymphocytes. Five healthy donors and 7 persons after in vivo exposure to 1.9-5.4 Gy from 2 to 48 years before investigation were studied. There were no clonal chromosome aberrations in bone marrow cells, peripheral blood lymphocytes and PBMD colonies derived from healthy donors. Results. Chromosome analysis of individual colonies was performed in 5 cases of irradiated persons. Frequency of colonies with unique clonal markers varies from 0 to 100% depending on exposure doses. In patients 1 and 2 (exposure dose - 3.8 and 5.4 Gy) colonies with different clonal markers were found, the same unique clonal chromosome markers were sometimes revealed in 2-3 colonies. During 2003-2005 years patient 2 was analyzed three times repeatedly and no colonies with the same aberration were found. Patient 3 (exposure dose - 3.6 Gy, 20 years ago) was analyzed only once and all available colonies showed bearing the same marker looking as del(22q) or t(5;22) (translocation t(5;22)) could not be excluded. In the other two patients (exposure dose - 1 and 2.3 Gy) colonies with clonal chromosome markers were not found. In peripheral blood lymphocytes of 4 patients stable chromosome aberrations were found in 22-85% of cells depending on irradiation dose. In two of them 9-15% of aberrations were clonal. From 4 to 8 clones were found in each case, some of the clones were large and represented 2-5% of evaluated cells. The indication of stable chromosome aberrations in T-lymphocytes can be explained by both a direct radiation effect on long-living lymphocytes and indirect cell defects originating from HSC. Summary/Conclusion. Our preliminary results suggest that hematopoiesis in humans is polyclonal. The size of the clones, their longevity and kinetics will be the subject of further investigation.

1065 EXCELLENT STEM CELL MOBILIZATION USING ESCALATED BEACOPP IN HIGH-RISK PATIENTS WITH HODGKIN’S DISEASE
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Introduction: After intensive treatment regimens have been established, the survival rate for patients with advanced Hodgkin’s disease is approximately 91% after five years and 13% of the patients have a relapse or have primary progressive disease (2%) within the first five years. For patients with relapse after conventional chemotherapy and radiation therapy, however, there is a real chance of achieving remission again. Since it is often difficult to harvest autologous stem cells following an intensive pre-treatment, our center embarks on the strategy to harvest autologous blood stem cells in high-risk patients, defined according to the risk stratification of the German Hodgkin’s Study Group, already as part of the initial polychemotherapy. Results. Between 9/2003 and 2/2006, we analyzed the results of the stem cell harvest of 14 consecutive patients with Hodgkin’s disease who were mobilized with the escalated BEACOPP regimen. There were 9 female and 5 male patients. Escalated BEACOPP was the primary therapy in twelve patients and a relapse was treated in two patients; the previous treatment was 4 or 6 cycles of the ABVD regime + involved field radiation. The twelve patients who did not receive previous treatment were classified as having an initial Ann Arbor stage II A/2 II B/5, III B/3 and IV B/2 and most of them had a large mediastinal bulk as an additional risk factor. The two patients who did receive a previous treatment were classified as having an initial Ann Arbor stage III A or IV B, without an additional risk factor. The stem cells were collected in 1 patient from cycle 2, in 9 patients from cycle 3 and in 4 patients from cycle 4 of the escalated BEACOPP regimen. A total of 13 patients received a standard dose of filgrastim, 5 μg/kg body weight s.c., from day 8 up to the last apheresis and 1 patient received pegfilgrastim 6mg s.c. All aphereses were performed using an Amicus cell separator™ (Baxter, MNC set, closed two-arm). 9 patients required only 1 apheresis and the remaining 5 patients required 2 aphereses. An apheresis result sufficient for a possible reinfusion could be achieved in all patients (4.26 - 14.4×106 CD34 cells/kg/body weight, mean: 7.7). Summary. According to our experience, escalated BEACOPP regimen is very suitable for the harvesting of stem cells in high-risk patients with Hodgkin’s disease even though the harvesting procedure with carboplatin. A sufficiently pure stem cells can also be collected from pretreated patients. The stem cell mobilization can be integrated into the escalated BEACOPP regimen safely and without a delay in treatment and thus creates, already at an early stage, the precondition for a high-dose therapy, which might be required in high-risk patients.

1066 MARROW CELLS CULTURED IN MSC MEDIUM EXPAND TO CD73, CD90 AND CD105 CELLS OF FIBROBLAST-LIKE MORPHOLOGY

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Backgrounds. Recent literature data suggest that in the marrow reside progenitors with a potential to regenerate not only hematopoietic sys-
HUMAN BONE MARROW ADIPOCYTES AND HEMATOPOIESIS: FROM UNILOCULAR FAT CELLS TO FIBROBLAST-LIKE FAT CELLS AND THEIR RELATION WITH CD34+ PROGENITORS DIFFERENTIATION IN THE ABSENCE OF EXOGENOUS CYTOKINES

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Backgrounds. The bone marrow microenvironment plays a critical role in regulating the growth and differentiation of hematopoietic cells. Both growth factors and cytokines, as well as direct cell-cell contacts, participate in these processes. Fat cells are heterogeneous present in the bone marrow and replace hematopoietic cells in bone marrow failure disorders. Thus, it is usually admitted that they play a passive role in hematopoiesis. Aim: In this work we have tested the hypothesis that adipocytes could play an active role in hematopoiesis. Methods: Adipocytes isolation, cell culture, RT-PCR, optic cytology, electronic microscopy, immunophenotypic analysis (FACS, confocal) and ELISA. Results: cocultures of FLFC and CD34+ positive cells, induce CD34+ differentiation essentially into macrophages (Mo) and dendritic cells (DC). Likewise, cocultures of FLFC and ELISA M-CSF, induce SCF, M-CSF and GM-CSF. In contrast, granulopoiesis was poorly represented and erythropoiesis was totally inhibited even in the presence of high dose of Erythropoetin (2U/mL). FLFC establish cell-cell contacts with Mo and DC, but this contact is however not critical since DC and Mo are obtained in transwell coculture. In contrast, in transwell experiments erythropoiesis and granulopoiesis were restored. Summary/Conclusion: Our data suggest that adipocytes have an active role in hematopoiesis. They may induce CD34+ cells differentiation towards Mo and DC, and inhibit through cell-cell contact, erythropoiesis and granulopoiesis. Our data may provide a new role for adipocytes in vivo, and may actively participate at the pathophysiology of bone marrow failure disorders. It remains to determine by which mechanisms FLFC operate in this process, to define new therapeutic strategies able to target FLFC.

HEMOLYTIC CRISIS DUE TO RASBURICASE IN A PATIENT WITH PREVIOUSLY UNKNOWN GLUCOSE-6-DH HYDROGENASE DEFICIENCY

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Glucose 6 phosphate dehydrogenase deficiency is a widespread congenital eritroenzymopathy, more frequent in individuals of African and Mediterranean ethnic origin. Hemolyis is arising by infections, certain foods and several drugs. Avoiding such precipitants is the only therapeutic measure. Rasburicase is a recombinant enzyme with urate oxidase activity employed for treatment of hyperuricemia and therapy. It is formally contraindicated in patients with decreased glucose 6 phosphate activity due to its oxidant activity capable of inducing haemolytic crises. We report the case of a 30-year-old male patient of African origin diagnosed of diffuse large cell B lymphoma stage III B affecting stomach and presenting as a massive gastric hemorrhage. At diagnosis bilirrubin was within normal limits and LDH was 1086 U/L. Anemia of 9,1 g/dL haemoglobin prompted red blood cell transfusion, then rising to 10,7 g/dL. Gastric hemorrhage precluded oral route for drugs, and allopurinol is not available in the intravenous form in our institution, so we started treatment with rasburicase once therapy with rituximab and CHOP was started. Once three doses of rasburicase had been administered the patient complained of dark urine, which turned to be black. In analysis anemia worsened suddenly falling to 6,9 g/dL; blood smears showed schistocytes and hematocrit was not previously noticed. In chemistry bilirrubin of 5,9 mg/dL, LDH of 4551 U/L and haptoglobin less than 6,5 mg/dL were the most important findings. A diagnosis of haemolytic crisis was made, rasburicase stopped and vigorous hydration with alkalinization instituted, as well as folic acid supplementation. Evolution was favourable, requiring transfusion of five red blood cell units. The patient was questioned for past haemolytic crises with negative results, since no episodes of dark urines were reported. Decreased glucose 6 phosphate dehydrogenase level despite previous blood cell transfusion was found, leading to the diagnosis of enzyme deficiency. Thus rasburicase caused the haemolytic crisis in this patient because of a previous enzyme deficiency. No haemolytic crises have appeared in subsequent courses of chemotherapy and rituximab. Rasburicase is formally contraindicated in glucose 6 phosphate dehydrogenase deficiency. This deficiency was suspected once hemolysis had started since no previous history of haemolytic crises had been reported. In patients of certain ethnic origin (African, Mediterranean) levels of glucose 6 phosphate dehydrogenase should be determined before administering treatment with rasburicase even without a previous history of haemolytic crises.

LOSS OF CASPASE-8 EXPRESSION DOES NOT CORRELATE WITH MYCN AMPLIFICATION, AGGRESSIVE DISEASE OR PROGNOSIS IN NEUROBLASTOMA

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Inactivation of caspase-8 because of aberrant gene methylation has been associated with amplification of the MYCN oncogene and aggressive disease in neuroblastoma suggesting that caspase-8 may function as tumor suppressor. However, the prognostic impact of caspase-8 in neuroblastoma has remained obscure. Therefore, we investigated caspase-8 expression and its correlation with established prognostic markers and survival outcome in a large cohort of neuroblastoma patients. Here, we report that loss of caspase-8 protein expression occurs in the majority (75%) of neuroblastoma and is not restricted to advanced disease stages. Surprisingly, no correlation was observed between caspase-8 expression and MYCN amplification in their mononuclear cells. Similarly, ectopic expression of MYCN or antisense-mediated downregulation of MYCN had no effect on caspase-8 expression in neuroblastoma cell lines. Also, caspase-8 expression did not correlate with other parameters of high-risk disease, e.g. Ip36 aberrations, disease stage, age at diagnosis or tumor histology. Most importantly, loss of caspase-8 protein had no impact on event-free or overall survival in the overall tumor population or in distinct subgroups of patients. By revealing no correlation between caspase-8 expression...
and MYCN amplification or other established parameters of aggressive disease, our findings in a large cohort of neuroblastoma patients demonstrate that inactivation of caspase-8 is not a characteristic feature of aggressive neuroblastoma. Thus, our study provides novel insight into the biology of this tumor, which may have important clinical implications.

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SENSITIZATION OF GLIOBLASTOMA CELLS FOR DEATH RECEPTOR- OR ANTICANCER DRUG-INDUCED APOPTOSIS BY PI3K INHIBITION

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Activation of the PI3K/Akt/mTOR pathway has recently been reported to correlate with increasing tumour grade, decreased apoptosis and adverse clinical outcome in human malignant glioma in vivo. However, the therapeutic potential of targeting the PI3K/Akt/mTOR cascade by kinase inhibitors for apoptosis sensitization of malignant glioma has not yet been investigated in detail. Here, we report that inhibition of PI3K by LY294002 significantly sensitized glioblastoma cells for death-inducing ligands (TRAIL, agonistic anti-CD95 antibodies) as well as for different anticancer drugs (Doxorubicin, Taxol, Vincriustin). In contrast to PI3K inhibition, blockade of mTOR by RAD001 (everolimus) or of MEK by UO126 did not significantly alter the sensitivity of glioblastoma cells for TRAIL- or Doxorubicin-induced apoptosis. Analysis of apoptosis pathways revealed that inhibition of PI3K resulted in downregulation of anti-apoptotic proteins such as FLIPs, XIAP, cIAP2 and survivin and cooperated with TRAIL or Doxorubicin to trigger loss of mitochondrial membrane potential, release of cytochrome c from mitochondria and full activation of the caspase cascade. Inhibition of caspases by the broad range caspase inhibitor ZVAD.fmk completely abolished apoptosis in response to combined treatment with LY294002 and TRAIL or Doxorubicin, indicating that apoptosis occurred in a caspase-dependent manner. By demonstrating that inhibition of PI3K significantly enhanced both death receptor- and anticancer drug-induced apoptosis in glioblastoma cells, our findings have important implications for the development of novel treatment strategies in glioma therapy. Thus, PI3K inhibitors represent a promising approach to enhance the antitumor activity of TRAIL or chemotherapy in glioblastoma.

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HBVAR-XPRBASE: A COMPREHENSIVE ONLINE REPOSITORY OF EXPERIMENTAL PROTOCOLS TO SCREEN FOR THE HUMAN GLOBIN GENE SEQUENCE VARIATIONS

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Backgrounds. Hemoglobinopathies, resulting from mutations in the α-, β-, or δ-like globin gene clusters, are the most common inherited disorders in humans, with approximately 7% of the world population being carriers of a globin gene mutation. Single nucleotide substitutions can lead to amino acid replacements that cause hemolytic anemias, such as sickle cell disease, or hemoglobinopathies that can minimally or drastically reduce their affinity. Molecular defects in either regulatory or coding regions of the human globin genes in adherent cell layers (ACL) of cultures from AA patients. PTH was added for 3 and 6 weeks of cultivation in concentration 10^{-5}, 5\times10^{-6} and 10^{-7} M once a week while changing half of the media. To characterize alterations in the expression of several genes in ACLs after PTH treatment semi-quantitative analysis of RT-PCR products was performed using PhosphoImager Cyclone, Packard Bell (USA) after Southern blot hybridization with appropriate sequences. The expression level of β-adactin was used as a normalization factor. Results. Osteoblastic cells activated by PTH produced high levels of the Notch ligand Jagged 1. Expression level of Jagged 1 increased insignificantly in donors’ ACLs after PTH treatment and did not change in AA patients’ ACLs. Notch ligand expression level in AA patients’ ACLs was stable and independent of PTH treatment or duration of cultivation. Expression level of Bmi 1 and Ang 1 genes taking part in regulation of HSC proliferation did not change in PTH-treated cultures from AA patients. Moreover, after 3 weeks in culture expression of Ang 1 was 5-fold lower in these cultures compared with donor ones. In donor ACLs PTH administration caused 3-fold increasing of Bmi 1 expression. Expression levels of cell adhesion molecules VCAM 1 and ICAM 1 in ACLs of both donors and AA patients were not sensitive to PTH treatment while in donor ACLs expression of ICAM increased significantly during cultivation. VEGF expression was markedly improved in donor ACLs after both PTH treatment and cultivation, whereas no changes in its expression were observed in AA patients ACLs. Summary/Conclusion. Stromal cells from patients with AA are not sensitive to PTH treatment. It may happen due to absence of activation of osteoblastic cells in their microenvironment after PTH administration and may point to pathology of these cells.

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ACUTE MYELOID LEUKEMIA: OUTCOME OF RELAPSE FOLLOWING ALLOGENEIC STEM CELL TRANSPLANTATION COMMENTS ON 46 CASES

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HBVAR-XPRBASE: A COMPREHENSIVE ONLINE REPOSITORY OF EXPERIMENTAL PROTOCOLS TO SCREEN FOR THE HUMAN GLOBIN GENE SEQUENCE VARIATIONS

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Backgrounds. Hemoglobinopathies, resulting from mutations in the α-, β-, or δ-like globin gene clusters, are the most common inherited disorders in humans, with approximately 7% of the world population being carriers of a globin gene mutation. Single nucleotide substitutions can lead to amino acid replacements that cause hemolytic anemias, such as sickle cell disease, or hemoglobinopathies that can minimally or drastically reduce their affinity. Molecular defects in either regulatory or coding regions of the human globin genes in adherent cell layers (ACL) of cultures from AA patients. PTH was added for 3 and 6 weeks of cultivation in concentration 10^{-5}, 5\times10^{-6} and 10^{-7} M once a week while changing half of the media. To characterize alterations in the expression of several genes in ACLs after PTH treatment semi-quantitative analysis of RT-PCR products was performed using PhosphoImager Cyclone, Packard Bell (USA) after Southern blot hybridization with appropriate sequences. The expression level of β-adactin was used as a normalization factor. Results. Osteoblastic cells activated by PTH produced high levels of the Notch ligand Jagged 1. Expression level of Jagged 1 increased insignificantly in donors’ ACLs after PTH treatment and did not change in AA patients’ ACLs. Notch ligand expression level in AA patients’ ACLs was stable and independent of PTH treatment or duration of cultivation. Expression level of Bmi 1 and Ang 1 genes taking part in regulation of HSC proliferation did not change in PTH-treated cultures from AA patients. Moreover, after 3 weeks in culture expression of Ang 1 was 5-fold lower in these cultures compared with donor ones. In donor ACLs PTH administration caused 3-fold increasing of Bmi 1 expression. Expression levels of cell adhesion molecules VCAM 1 and ICAM 1 in ACLs of both donors and AA patients were not sensitive to PTH treatment while in donor ACLs expression of ICAM increased significantly during cultivation. VEGF expression was markedly improved in donor ACLs after both PTH treatment and cultivation, whereas no changes in its expression were observed in AA patients ACLs. Summary/Conclusion. Stromal cells from patients with AA are not sensitive to PTH treatment. It may happen due to absence of activation of osteoblastic cells in their microenvironment after PTH administration and may point to pathology of these cells.

1074
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Acute myeloid leukemia (AML) patients relapsing after allogeneic stem cell transplantation (allo-Tx) have a very poor prognosis. Discontinuation of immunosuppression and donor lymphocyte infusion exhibit activity against leukemic cells. However, long-term outcome is disappointing. Patients’ selection for salvage treatment is very important. Forty-six consecutive AML patients relapsed after allo-Tx between 9/1992 and 7/2005. Age at transplantation ranged between 15 and 60 years (median, 36). Eleven patients were in early, 14 in intermediate, and 19 in advanced stage at the time of transplantation. Donors were HLA identical siblings (n=34), mismatched related (n=6) and matched unrelated (n=6). In all but one case ablative conditioning was used. In all but one (haploidentical sib with 5 HLA Ag m/m), cases T-cell repleted grafts were given. Relapse occurred 1 to 90 months after transplantation (median, 3). Eight patients had an early relapse (<30% blasts in BM), and two other had exclusively extramedullary relapse. Salvage therapy was introduced in 30 of the 46 patients and included: Combined chemotherapy (n=5), donor lymphocyte infusion (n=5), combined chemotherapy or serotherapy plus donor lymphocytes or haemopoietic cells (n=11), high dose chemotherapy or serotherapy plus haemopoietic cells (n=9). One patient suffering from acute promyelocytic leukemia was treated with As2O3 among other modalities. Eleven patients died due to therapy related toxicity (57%). Complete remission was achieved in 15 patients (50% of the patients receiving salvage therapy and 33% of the total relapsed patients). Four patients are alive and in CR for 3, 22, 40, and 53 months after relapse. In two out of these four patients acute and subsequently progressive chronic graft versus host disease developed for the first time after relapse. Overall survival for all patients is 12.7% at 4 years. Among eight patients with early relapse, 5 are alive and in CR for 3, 22, and 53 months. Both patients with extramedullary relapse are alive but still with relapsing extramedullary disease for 14 and 29 months. In conclusion, the very poor prognosis of relapse after allo-Tx in AML is confirmed. Even in selected patients salvage therapy is accompanied with high mortality rate. Nevertheless, it must be emphasized that in a small proportion of patients long and disease-free survival is achievable. A possible survival advantage of patients with early relapse (i.e. >30% blasts in BM) points out to the importance of close follow-up after Tx. Long survival (even not disease-free) of patients with extramedullary relapse is remarkable and the involved mechanisms deserve further studies.

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**USE OF FROZEN EMBRYOS FOLLOWING STEM CELL TRANSPLANTATION FOR LEUKEMIA: A SINGLE CENTRE EXPERIENCE.**

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Infertility is common following stem cell transplantation (SCT) for leukemia. Options for parenthood need to be considered before high dose chemotherapy and total body irradiation are given and include cryopreservation of fertilised embryos, oocytes (unfertilised mature eggs) or ovarian tissue. The pregnancy rate per embryo transfer in otherwise healthy women is approximately 25% with a take home baby rate of approximately 16-18%. The success rate using frozen embryos in women who have received chemoradiotherapy as treatment for cancer is not known. There are theoretical concerns that success will be lower in these patients compared to the normal population because of the effects of chemoradiotherapy on the uterus. Pre-transplant chemotherapy or disease may also have deleterious effects on the female reproductive system. At the IVF Unit, Hammersmith Hospital, 6 women with an underlying diagnosis of CML have attempted pregnancy using embryos cryopreserved prior to SCT.

**Table 1. Outcome of frozen embryo transfer.**

<table>
<thead>
<tr>
<th>Treatment cycles</th>
<th>Pregnant/ not pregnant (NP)</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>n=3</td>
<td>P</td>
</tr>
<tr>
<td>Patient 2</td>
<td>n=1</td>
<td>P</td>
</tr>
<tr>
<td>Patient 3</td>
<td>n=1</td>
<td>NP</td>
</tr>
<tr>
<td>Patient 4</td>
<td>n=2</td>
<td>NP</td>
</tr>
<tr>
<td>Patient 5</td>
<td>n=2</td>
<td>P</td>
</tr>
<tr>
<td>Patient 6</td>
<td>n=2</td>
<td>NP</td>
</tr>
</tbody>
</table>

Of these 6 women, 5 had allogeneic SCT with conditioning which included total body irradiation and one had an autologous SCT conditioned with high dose busulphan only (patient 4). The results are tabulated below. Eleven treatment cycles using cryopreserved embryos in 6 women led to 4 pregnancies in 3 women. Of these, two pregnancies have been successful. There have been two miscarriages, one (patient 1) at 12-13 weeks gestation and the second (patient 5) at 7 weeks gestation. We compared the success of using the frozen embryos with the outcomes of pregnancy following SCT. Despite the low pregnancy and low take-home baby rate, cryopreservation of embryos prior to SCT remains the best option for women likely to develop treatment-related ovarian failure and who wish to have their own genetic offspring following SCT.
B with >7 log·copies/mL HBV DNA and 1-36 fold (median 4) ALT increase. Two patients developed jaundice but none developed clinical decompensation, no patients required hospitalisation. Two patients were treated with lamivudine 100 mg/daily; one patient’s ALT levels and serum HBV-DNA progressively declined whereas the other patient developed lamivudine-resistance, requiring additional treatment with adefovir dipivoxil. Three out of 4 untreated patients maintained persistently high levels of serum HBV-DNA and abnormal ALT throughout the study period, whereas one lost HBsAg and developed anti-HBs. Reverse HBsAg seroconversion occurred in 4 out of 22 (18%) patients who developed chronic graft-versus-host disease (cGVHD) as compared to 2/18 (11%) who did not. Conclusion. The high rate of HBV reverse seroconversion and the development of chronic hepatitis B in all these patients call for a prophylactic anti-HBV treatment in all HBsAg negative/anti-HBc positive patients undergoing HSCT.

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ANGIOGENIN LEVELS IN PATIENTS WITH POLYCYTHEMIA VERA
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Angiogenin is a protein with a potent function in angiogenesis, which circulates in human serum and is secreted by haematopoietic cells, endothelial cells, vascular smooth cells and fibroblasts. Its serum levels are increased in patients with solid tumors, acute myeloid leukemia, myelodysplastic syndromes, chronic myeloid leukemia and essential thrombocytosis. No data are available about angiogenin levels in patients with polycythemia vera. In this study we aimed to evaluate the levels of angiogenin in serum of patients suffering of polycythemia vera and examine any possible correlation with bone marrow microvascular density (MVD) determined by CD34 count on bone marrow trephines. A total of 29 patients with PV (14 males and 15 females) with a mean age of 57 ± 15.4 (m ± SD) years (range 24-81) were included. The control group consisted of 16 healthy subjects (8 males and 8 females) with a mean age of 55.9 ± 6.7 years (range 46-71). Serum levels of angiogenin were measured by a commercial quantitative sandwich enzyme immunoassay. In twenty four of them we estimated the MVD in bone marrow samples immunostained with anti-CD34 monoclonal antibody by counting the number of vessels per 400x high power field (HPF) using light microscopy. Serum angiogenin concentrations were found to be significantly higher in polycythemic patients than in the control group (48±145 pg/mL and 540±69 pg/mL, respectively, p=0.037). In the patient and in the control group we found no statistically significant correlation between serum angiogenin levels and platelet counts, haemoglobin, WBC counts and age. No difference was found between patients angiogenin levels on different therapeutic regimens. The microvessel density of the bone marrow of the 24 polycythemic patients was found to be 7.1±4.1 vessels per HPF and significantly elevated in comparison to normal bone marrow specimens (n=10, MVD: 2.0±1.6, p=0.01). Interestingly a negative correlation was found between serum angiogenin levels and the microvessel density of the bone marrow (r=-0.49, p=0.085). In the present study, serum angiogenin levels were found to be significantly increased in patients with PV in comparison to the control group. To our knowledge there is evidence of pronounced angiogenin increase in PV by few reports on other angiogenic factors as vascular endothelial growth factor and basic fibroblast growth factor. In addition the current study demonstrated increased MVD in comparison to healthy control group indicating augmented angiogenic procedure. The observation that MVD is negatively correlated with angiogenin serum levels, although at first sight unexpected, could be explained by the hypothesis that in patients group the less bone marrow vascularity acts modulating the increase of angiogenin production which in turn enhances the bone marrow vascular expansion. It is emphasized that this observation should be confirmed by other studies as well. The exact contribution of angiogenin in the pathophysiology of PV and its prognostic significance as disease activity marker deserves further research.
range 17-63 years) with ET (n=17), PV (n=5), IMF (n=14) the mean levels in blood serum were significantly higher (19±1.7 µmol/L) in comparison with 40 donors in control group (12±1.3 µmol/L, p<0.00002). In the group with thrombotic episodes the mean level in the patients with IMF was much higher (26±4.7 µmol/L) than for patients with ET (20±3.8 µmol/L) and PV (23±5.9 µmol/L), p<0.002. Despite of different frequency of alleles 677 of MTHFR gene in patients with MPD, HHC occurred with the same frequency in all groups, which was different from the situation in healthy people. We did not find out a significant relationship between MTHFR genotype and the rate of thrombotic complications. For MPD patients with normal and elevated HC concentration in blood serum it was shown, that factor VIII level was higher in HHC, than in patients with normal HC level (222±26.5% and 116±20%, respectively, p<0.002). The same was found for von Willebrand factor (202±15.6, 120±14.6%, p<0.003). Comparing the data on ADP induced platelet aggregation in patients with thromboses, it was shown that the activation of the later was higher in HC against normal data of blood HC (77 ±15.7% and 47±12.1%, accordingly, p<0.001). Regressional analyses showed that only HHC has a statistically significant influence on thrombotic complications rate for MPD patients (p=0.004). We consider that the lowering of HC plasma levels together with vitamin therapy were the more expressed, the higher was its baseline level. Conclusion. The relationship between HHC levels and thromboses in the patient’s group proves that different stages of myeloproliferative diseases may influence the HC levels in patients with MPD. Homocystein is a highly significant, independent risk factor for thrombotic complications. Therefore it is necessary to discover and treat HHC.

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Background. The chronic myeloproliferative diseases, polycythemia vera (PV), essential thrombocythemia (ET) and idiopathic myelofibrosis (IMF) share more similarities with each other and often difficult to distinguish from other causes of elevated blood cell counts. These diseases are clonal, arising from a single hematopoietic stem cell or progenitor and lacking of specific biological markers. Despite the clinical and pathologic features, the diagnosis of an individual patient with isolated erythrocytosis or thrombocytopoiesis is often difficult. Aims. The aim of this study was to compare the serum erythropoietin (EPO) level and the endogenous erythroid colonies (eBFU-E) in semi-solid cultures of bone marrow and peripheral blood cells in patients with PV, ET and IMF. Methods. EPO level was determined in serum of 22 PV patients, 22 ET patients and 8 IMF patients at diagnosis and 16 PV, 19 ET and 8 IMF during/after the treatment. The growth of eBFU-E was evaluated in vitro in bone marrow and peripheral blood in 41 PV patients, 38 ET patients and 12 IMF patients at diagnosis and during/after the treatment. Results. In PV 77% patients at diagnosis and 62% patients receiving myelosuppressive therapy EPO level was below the normal limits. 85% untreated ET patients and 60% treated with myelosuppressive agents had subnormal EPO level. The low EPO values were detected in 87% untreated IMF patients and in 57% patients during/after treatment. Nineteen (95%) of 20 PV patients presented eBFU-E growth in bone marrow, and in 13 (72%) of 18 patients showed circulating eBFU-E at diagnosis but only 2 (16%) of 12 PV patients had bone marrow eBFU-E and in 13 (51%) of 24 patients circulating eBFU-E were observed after the treatment. In untreated ET patients 3 (53%) of 9 showed bone marrow eBFU-E and 3 (27%) showed eBFU-E in peripheral blood; in 5 (20%) of 24 ET patients in bone marrow and in 11 (29%) of 38 patients in peripheral blood eBFU-E were detected after the treatment. Among IMF patients, 3 (75%) of 4 were positive for bone marrow eBFU-E and all patients had eBFU-E in peripheral blood at diagnosis; 4 (66%) of 6 patients IMF and 4 (40%) of 10 patients had eBFU-E in bone marrow and peripheral blood, accordingly. Thus the low serum EPO level and the growth of eBFU-E in cultures of bone marrow and peripheral blood were maintaining in the most of PV, ET and IMF patients. Correlation was observed between EPO and endogenous growth of BFU-E in 5 (71%) of 7 PV patients in bone marrow and in 9 (47%) of 19 patients in peripheral blood. In ET patients the correlation was observed in 6 (53%) of 11 patients in bone marrow and in 10 (67%) of 15 patients in peripheral blood. Significant correlation between EPO and eBFU-E in IMF was found in 50% patients both in bone marrow and in peripheral blood. Conclusions. We conclude that EPO level and the growth of eBFU-E in vitro are useful in the diagnosis and monitoring of minimal residual disease of polycythemic conditions and diseases with increased platelet levels. The investigation of anomalous erythroid proliferation and regulation in patients with chronic myeloproliferative diseases were useful and reliable tools for the diagnosis and pathogenesis of these diseases.

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JAK2 V617F MUTATION AND CLINICAL BEHAVIOR IN PATIENTS WITH ESSENTIAL THROMBOCYTOMIA: RESULTS FROM A SINGLE CENTER STUDY

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It has been reported that an acquired V617F mutation in JAK2 occurs in approximately 50-80% of patients with essential thrombocytemia (ET). Recent reports suggest that the presence of this mutation is associated with clinical features resembling polycythemia vera as well as an increased risk of thrombosis. Aim: the aim of present study was to verify whether patients suffering from ET and positive for JAK2 V617F mutation (mutation-positive) display a distinct clinical behavior from those without JAK2 V617F mutation (mutation-negative). Methods. Genomic DNA from peripheral blood granulocytes of patients with ET attending the outpatient Clinic were tested for the presence of JAK2-V617F mutation by an allele-specific Polymerase Chain Reaction (Raucher E et al. Lancet 2005;365:1054-61); their clinical records were reviewed for their diagnostic blood counts, thrombotic histories and bleeding events. Thrombotic complications included major thromboses as well as microvascular disturbances. Results: among the 53 ET patients in who was possible to assess the status of JAK2 V617F mutation, 23 (67.9%) were mutation-positive and 10 (30.3%) were mutation-negative. Blood counts at diagnosis and relevant clinical events are summarized in Table 1.

<table>
<thead>
<tr>
<th>Number (%)</th>
<th>All patients</th>
<th>Mutation positive</th>
<th>Mutation negative</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female/male</td>
<td>21 (12)/216</td>
<td>12 (7)/104</td>
<td>9 (5)/112</td>
<td>0.03</td>
</tr>
<tr>
<td>Age at diagnosis (years)</td>
<td>60 (NS)</td>
<td>64 (NS)</td>
<td>56 (NS)</td>
<td>0.01</td>
</tr>
<tr>
<td>Disease duration (months)</td>
<td>68 (NS)</td>
<td>67 (NS)</td>
<td>71 (NS)</td>
<td>0.03</td>
</tr>
<tr>
<td>WBC (×10³/µL)</td>
<td>14.1 (±1.1)</td>
<td>14.7 (±1.2)</td>
<td>13.6 (±1.4)</td>
<td>0.03</td>
</tr>
<tr>
<td>Platelets (×10¹²/µL)</td>
<td>503 (±87)</td>
<td>503 (±87)</td>
<td>503 (±87)</td>
<td>0.83</td>
</tr>
<tr>
<td>Subjects with thrombosis (%)</td>
<td>14 (42%)</td>
<td>9 (39%)</td>
<td>5 (50%)</td>
<td>0.05</td>
</tr>
<tr>
<td>Subjects with bleeding (%)</td>
<td>7 (21%)</td>
<td>5 (22%)</td>
<td>2 (20%)</td>
<td>NS</td>
</tr>
<tr>
<td>Subjects with microvascular events (%)</td>
<td>8 (24%)</td>
<td>7 (30%)</td>
<td>1 (10%)</td>
<td>NS</td>
</tr>
<tr>
<td>Patients receiving cytoreduction (%)</td>
<td>28 (85%)</td>
<td>22 (96%)</td>
<td>6 (60%)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Mutation-positive patients were significantly older than mutation-negative; in mutation-positive patients blood counts at diagnosis were higher than in mutation-negative individuals, with a statistically significant difference for hemoglobin concentration. With a similar median follow-up of 67 and 71 months - respectively for mutation-positive and mutation-negative patients, no difference was observed in the occurrence of thrombotic or bleeding events between the two groups of patients, while a significantly higher number of mutation-positive patients required some form of cytoreduction at any time from the diagnosis. Conclusions: our study suggests that the presence of the JAK2 V617F mutation, particularly frequent in our series of ET patients, may identify a subgroup of patients with a more pronounced polycythemic behavior, as already suggested.
CLINICAL COURSE OF 65 PATIENTS WITH MYELOFIBROSIS WITH MYELOID METAPLASIA: EXPERIENCE OF MONZA CENTER

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Backgrounds. Myelofibrosis with myeloid metaplasia (MMM) is a chronic myeloproliferative disorder, characterized by bone marrow reactive fibrosis, extramedullary hemopoiesis, progressive anemia and marked splenomegaly. Overall median survival ranges from 3.5 to 5,5 years, according to the presence or absence of adverse prognostic factors, with final evolution toward disease progression (DP) or leukemic transformation (LT). Aim and Methods. We analysed 65 MMM patients (pts) referred in our hematology unit from 1999 to 2005, in order to provide information about initial features, treatment, clinical course and survival. Results. The median age at diagnosis was 66 years (range 59-79) with 17 pts (26%) aged less than 55 years and a M/F ratio of 44/21. 39 pts (60%) presented Idiopathic MMM, 26 (40%) MMM secondary to Polycythemia Vera (7) or Essential Thrombocythemia (19). At diagnosis, spleen enlargement (median 5 cm, range 1-30) below costal margin was present in 52 pts (80%). The median value of WBC was 11,9×10^9/L, of Hb was 11,6 g/dl, of platelets was 358×10^9/L. 45 pts (74%) had circulating myeloid precursors; 21 (32%) pts had blasts. The median value of LDH was 531 U/L and the median count of CD34+ cell was 59,8×10^9/L (evaluated on 37 pts). According to disease status, 18 pts (28%) received no treatment, 13 (20%) supportive care alone, 9 (14%) androgens or steroids, 23 (35%) anti-platelet drugs and 27 (41,5%) myelosuppressive agents alone or in combination with the above treatment. Eight pts (12%) underwent splenectomy after a median of 11,5 months from diagnosis. Only 3 pts underwent allogeneic stem cell transplantation. 50 pts (77%) are actually alive after a median follow-up of 28 months (range 7-134), of whom 16 (25%) died due to disease progression and 34 (52%) due to other causes. Nineteen pts (29%) died due to disease progression, 10 pts (15%) due to other causes and 15 pts (23%) due to other causes. Conclusion. Our experience confirms different outcome of MMM pts according to Dupriez score. Younger patients have a longer median survival. DF and LT correlate with increase of spleen volume, onset of constitutional symptoms and higher LDH levels with respect to diagnosis.

THE CD44 MAB A3D8 INDUCES APOPTOSIS AND G1 CELL CYCLE ARREST IN NEOPLASTIC MAST CELLS IN SYSTEMIC MASTOCYTOSIS

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Backgrounds. Recent data suggest that CD44 may serve as a new therapeutic target in AML and possibly also in other myeloid neoplasms. Systemic mastocytosis (SM) is a myeloid neoplasm characterized by abnormal growth and accumulation of mast cells (MC) in one or multiple organs. We have previously shown that normal tissue MC express CD44. Aims. In the present study, we asked whether CD44 is expressed on neoplastic human MC and whether CD44 ligation by the monoclonal antibody (mAb) A3D8 would be associated with inhibition of growth of neoplastic MC. Methods and Results. As assessed by flow cytometry, primary neoplastic MC were found to express CD44 in all patients with SM studied and a significantly elevated median number of circulating CD34, CD33, CD33/34 co-expression on CMD patients cells. Results. Analysis of the six patients with neoplastic MC obtained from a patient with MCL (A3D8, 5×10^6/L) and in a group of patients with systemic mastocytosis (SM), marked and sustained eosinophilia is detectable (SM-eo). Although the molecular defect has been defined for some of these patients, little is known about the impact and clinical correlates of eosinophilia in SM. In a cohort of 61 patients with SM, we identified 11 with persistent eosinophilia (>1,500/µL). According to the WHO-classification, 4 had indolent SM (ISM), 1 smouldering SM (SSM), 2 SM with associated chronic eosinophilic leukemia (SM-CEL), and 4 aggressive SM (ASM). Results. In the 2 patients with SM-CEL, the FIP1L1/PDGFRA fusion gene-product was detectable, but no KIT mutation at codon 816 was found, whereas in most other SM-eo patients, KIT D816V, but not FIP1L1/PDGFRA, could be detected. Other molecular defects including BCR/ABL, CBFA2/TAF1, JAK2 V617F, or a monoclonal T cell receptor rearrangement were not detected in patients with SM-eo. In the two patients with SM-CEL, fatal organopathy of the heart developed. By contrast, in all other SM-eo patients, organopathy, if recorded, affected the bone marrow, liver, or skeletal system, but did not affect the heart, even if eosinophilia persisted for many years. Conclusions. Our data show that the biochemical basis of eosinophilia in SM is variable and correlates with organopathy. SM-eo thus is a prediagnostic checkpoint but not a final diagnosis. For correct final diagnosis and selection of targeted drugs, it is important to apply molecular markers including FIP1L1/PDGFRA in SM-eo.

DIAGNOSTIC AND CLINICAL RELEVANCE OF CHRONIC MYELOPROLIFERATIVE DISORDERS PERIPHERAL CELLS ANTIGENS

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Backgrounds. Immunophenotype of peripheral blood cells in patients with chronic myeloproliferative disease (CMD) has not been extensively investigated to recognise association between CMD subtype, phase of disease and peripheral cells antigens. Aim. The aim of our study is the identification of same cytofluorimetry parameters useful to follow up patients with CMD. Methods. We analyzed the immunophenotype of 20 consecutive patients observed in our institute, during cytoreductive treatment for CMD. Of 20 patients 9 had essential thrombocythemia (ET), 7 myelofibrosis (MF) and 4 atypical myeloproliferative disorders (AMD). Flow cytometry was performed on peripheral blood sample using double or triple platform assay to identify CD34, CD38, CD61, CD42a positive and CD33/34 co-expression on CMD patients cells. Results. The main hematologic characteristics analyzed in CMD patient about white blood cells and platelet count were reported in ET group WBC 6.9-16.1×10^9/L, Plt 457-1976×10^9/L, in AMD group WBC 4.26-9.8×10^9/L, Plt 171-357×10^9/L, in MF group WBC 8.53-26.4×10^9/L, Plt 9-1595×10^9/L. The median of circulating CD34+, CD38, CD61, CD42a, expressed in 106 cells were in ET group (20, 117, 0, 463, 469 respectively), in AMD group (54, 79, 4, 553, 435 respectively), in FML group (63, 261, 33, 145, 371 respectively). We observed higher expression of CD38 than CD34 in CMD patients studied and a significantly elevated median number of circulating CD38 and CD34 in patients with MF, especially not responsive to cytoreductive treatment and inverse relationship between absolute number of CD33 positive and platelet count. Instead we evaluated a statistical correlation between of CD 61 and 42a positive cells and thrombocytosis in ET and AMD groups than others CMD patients. Summary/Conclusions. These initial data reflect the increased number of circulating CD38, CD34 and CD42a positive cells in patients with MF not responsive to treatment. This correlation may show an abnormal function of bone marrow for a hematopoietic differentiation decline. Further studies with a larger number of patients could improve the identification of surface antigens to classify better subclass of CMD associated to clinical features and to predict prognostic evolution of disease.
TREATMENT WITH HYDROXYUREA AS SINGLE AGENT DOES NOT INCREASE THE RISK OF SECOND MALIGNANCIES IN ESSENTIAL THROMBOCYTHEMIA PATIENTS

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Among chronic myeloproliferative disorders, Essential Thrombocythemia (ET) has the most favourable prognosis, with thrombotic events and second malignancies representing major causes of death. Several agents have been well tolerated and used in the management of ET. Among them, hydroxyurea (HU) is the most commonly used cytoreductive agent in ET patients, improving thrombohemorrhagic complications and clinical outcomes. However, the effect of HU on the risk of second malignancies is not well established.

Aims. We have investigated the risk of second malignancies in ET patients treated with HU as single agent.

Methods. Retrospective analysis of a large population of ET patients with long-lasting observation times, showing that treatment with single agent HU does not increase the risk of the second malignancies, in particular of haematological subtype. On the contrary, our results confirm the notion that cumulative high dose of ALK is associated with high risk of developing second cancers.

References
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3. Pettit RM. Presented at the 45th Annual Meeting of the American Society of Hematology. December 6-9, 2005, San Diego, California, USA.

Assessing the Benefit/Risk Balance of Long-Term Anagrelide Hydrochloride (Xagrid/Agrilin) Treatment for Essential Thrombocythemia

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Backgrounds. Essential thrombocythemia (ET) is a myeloproliferative disorder (MPD) associated with thrombohaemorrhagic complications. The disease uncommonly progresses to myelofibrosis or acute leukaemia; the risk of transformation may be increased by cytoreductive treatments. Anagrelide (Xagrid/AgrilinTM) is a non-cytotoxic agent that selectively reduces platelet production. Its long-term efficacy and safety in patients with MPD (maximum duration of exposure, 7.1 years) have been previously described.1 Aims. To assess the benefit/risk balance of long-term anagrelide therapy in patients with ET. Methods. Retrospective analysis of an open-label, multicenter anagrelide trial. Results. Data from 5660 patients with MPD (2251 with ET) were included in the safety analysis. The maximum duration of follow-up was 11.4 years. Prior myelosuppressive therapy had been administered in 81% of patients (reasons for change to anagrelide, toxicity [33%] and poor platelet control [31%]). Efficacy data were available for 954 patients with ET. A response rate of 78.7% was observed (67.2% complete responses [decrease in platelet count to ≤500×10^9/L, or a decrease of ≥50% from baseline within 4 weeks of the start of anagrelide therapy]; 11.5% partial response [decrease of 20% to <50% from the baseline value at least 4 weeks after starting anagrelide]).2 Response rates for patients who had failed previous therapy and for patients who were intolerant of previous therapy were similar (78.8% and 78.6%, respectively). After the first year, platelet count decreases were well maintained. Results were similar for both sexes, different age groupings, and ethnic origins. At baseline, 163/994 (17.5%) patients reported ET-related symptoms, including GI and other bleedings, arterial or venous thromboses, angina, pulmonary embolism, transient ischaemic attacks, peripheral ischaemia, and paraesthesia.3 This had reduced to 7.9% (63/796, p<0.001) after 12 weeks and was maintained during follow up (3.2% [15/470] at 1 year and 2.5% [6/239] at 2 years, p<0.001). Adverse events occurred in 40.2% of the patients and were generally mild. Anagrelide was discontinued in 38.6% of patients (adverse events accounting for 29.2% of patients stopping treatment). Transformation to AML/MDS occurred in 47/2251 (2.1%), but only in subjects who had previously been exposed to cytoreductive treatment. The observed mortality rate (8.8%) was consistent with that which would be expected in ET patients. The most common reasons for death (≥1%) were CML, reason unknown or unspecified, and sepsis. Summary/Conclusions. Anagrelide effectively reduces platelet counts and thrombohaemorrhagic complications in patients with ET. This is independent of gender, age, ethnic origin, and prior therapy. The drug demonstrates a recognized safety profile. Benefits are maintained during long-term follow-up without an increase in disease transformation.

References
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The Combination of Fludarabine, Ara-C, Idarubicin and GEMTUMUZUM-OZOGAMICIN (MY-FLA) IS A SAFE AND EFFECTIVE THERAPY FOR ELDERLY AML PATIENTS


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Background. Elderly AML patients and patients with AML evolved from MDS or therapy related displayed a very poor prognosis. In the last decade the association of fludarabine, Ara-C and anthracycline proved to be an effective and well tolerated induction regimen for this group of patients and, more recently, the introduction of gemtuzumab ozogamicin has opened new perspectives in the treatment of AML. Methods. We report here our preliminary experience on 18 elderly AML patients treated as first line therapy with MY-FLA regimen (Fludarabine 25 mg/m2, Ara-C 1 g/m2, idarubicin 5 mg/m2 all for 3 days, followed by gemtuzumab ozogamicin 5 mg/m2 at day 4). Responding patients received the same as first line therapy with MY-FLA regimen (Fludarabine 25 mg/m2, Ara-C 1 g/m2, idarubicin 5 mg/m2 all for 3 days, followed by gemtuzumab ozogamicin 5 mg/m2 at day 4). Responding patients received the same regimen as consolidation therapy. Results. The median age of patients was 66 (range 54-76); M/F ratio was 8/10; FAB subtypes were M0 in 1 patient, M1 in 8, M2 in 5, M4 in 2, M5 in 1, M6 in 1. Nine patients had de-novo AML (50%); in nine patients AML was secondary to NHL (3), MDS (4), epithelial neoplasms (2). Thrombohemorrhagic parameters before therapy were the following: WBC 6×10^9/L (range 1.7-110); Hb 9.4 g/dL (7.3-12); PLT 40×10^9/L (15-190). Cytogenetic analysis revealed a poor prognosis alteration in 9 patients (50%, with 7 complexes karyotypes) and an intermediate alteration in the other 9 patients. Results. The neutrophil (PMN > 0.5×10^9/L) and platelet (>25×10^9/L) recovery required a median of 18 (range 11-23) and 18 days (range 10-29) from the end of therapy. The median number of days with fever (>38°C) was 5. Therapeutic toxicity consisted of grade III-IV mucositis in 7 patients, grade III-IV sepsis in 4, grade IV febrile neutropenia in 2, grade II-III thrombocytopenia in 6, grade III-IV anemia in 1 patient. Nausea and vomiting occurred in 5 patients (27.8%) among patients who received only ALK, and 10 cases (25%) among patients treated with ALK+HU. Therefore, the proportion of second malignancies was higher (p=0.01) in patients who received ALK as part of their treatment, whereas no significant difference was documented between patients who did not receive any treatment and patients who received HU as single agent. By multivariate analysis, high dose of Melphalan was statistically different with respect to no use of Melphalan (Wald test=12.1 p value=0.005, odds ratio 5.9 with 95% confidence interval from 1.08 to 12.47). HU was not significant with both doses. Use of HU after ALK was not significant. Similar findings were observed when statistics were applied only to patients with haematological malignancies. This study, derived from a large population of ET patients with long-lasting observation times, shows that treatment with single agent HU does not increase the risk of the second malignancies, in particular of haematological subtype. On the contrary, our results confirm the notion that cumulative high dose of ALK is associated with high risk of developing second cancers.

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EFFICACY AND SAFETY OF BORTEZOMIB IN PATIENTS WITH REFRACTORY AND RELAPSED MULTIPLE MYELOMA OUTSIDE CLINICAL TRIALS: RESULTS FROM THE CATALAN MYELOMA/AMYLOID STUDY GROUP (GEMMAC) IN 120 PATIENTS

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Background. Bortezomib (Velcade) has recently been approved for the treatment of refractory and relapsed multiple myeloma (MM). In this setting, a response rate ranging from 35 to 50% has been reported in patients included in prospective clinical trials. However, the data on the efficacy and safety of bortezomib outside the context of clinical trials are limited. Aim. To analyze the efficacy and safety of bortezomib therapy in refractory or relapsed MM patients treated in community practice.

Patients and Methods. Between August 2003 and February 2006, 120 patients (63M/57F, median age: 63 years) with refractory or relapsed MM were treated with bortezomib outside the context of clinical trials in 16 centers in the area of Catalonia. Fifty-five (46%) patients had untreated relapse, 38 (32%) refractory relapse and 27 (23%) primary refractive disease. Twenty-seven of them (22%) had extramedullary plasmacytomas. The median number of previous lines of therapy was 2 (range: 1-6). Forty-two patients (42%) had received high dose therapy followed by stem cell transplantation (HDT/SCT); single autologous (55), double autologous (6), autologous followed by allogeneic (12), double autologous (6), autologous followed by allogeneic with reduced-intensity conditioning (6) and allogeneic (3). Bortezomib was administered intravenously at a dose of 1.3 mg/m2 on days 1, 4, 8, and 11 of every 21-day cycle. Six patients who had no response after two cycles of bortezomib alone continued treatment receiving also oral dexamethasone. The median number of cycles administered was 3.5 (range: 1-13). At the time of this analysis, bortezomib therapy was still ongoing in 22 cases, and 12 patients were not yet evaluable for response. Responses were evaluated according to the EBMT criteria. Results. Among the 108 already evaluable patients, the response rate to bortezomib was 52% (57/108), with 7 (6%) complete, 38 (35%) partial and 12 (11%) minimal responses. The remaining 51 patients showed no response: no change (57/108), with 7 (6%) complete, 38 (35%) partial and 12 (11%) minimal responses. The median time to best response was 3.5 months (range: 0.5-11). Grade 3 or 4 adverse events, which occurred in 45% of evaluable patients, included: thrombocytopenia (30%), asthenia (12%), peripheral neuropathy (9%), gastrointestinal symptoms (4%), fever (4%), postural hypotension (2%), rhabdomyolysis (1%) and tumour lysis syndrome (1%). The drug was discontinued because of side effects in 10 patients; peripheral neuropathy (7), asthenia (1), thrombocytopenia (1) and unknown (1). After a median follow-up of 7.4 months (range: 1.6-50), 15 of the 57 responding patients had relapsed (26%). Conclusion. In this observational study, the response rate to bortezomib in patients with relapsed and refractory MM treated in community hospitals was comparable to that achieved in the recently reported prospective clinical studies. Toxicity was manageable, but led to bortezomib discontinuation in 10% of the patients. In the present series, the evaluation of time to progression and overall survival requires longer follow-up.

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INHERITED COAGULATION DISORDERS IN CENTRAL PART OF IRAN

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Backgrounds. The incidence of hereditary coagulation disorders may vary according to the country and ethnic origin. Demographic datasets are vital in setting priorities, allocation of resources, measurement of outcomes, and comparison of alternate approaches. Aim: The aim of this study was to document the epidemiological features, disease severity and complications associated with inherited coagulation disorders in central part of Iran. Methods. A comprehensive survey was undertaken in January 2006. Clinical history, Laboratory and treatment data, and long term complications of all cases (553 persons) diagnosed with inherited coagulation disorders, were studied in Hematology-Oncology Department, Isfahan University of Medical Sciences. Results. 465 male and 88 female with Mean±SD age of 25.4±12.9 were studied. Hemophilia A was found in 341(61.7%), 48 (8.7%) had hemophilia B, 74 (13.4%) had Von Willebrand disease, and 34(6.1%) had platelet dysfunctions. The rare coagulation disorders (n=88) include 30 patients with FV deficiency, 23 with FVII, 13 with afibrinogenemia, 10 with FX. Among them 19 (9.4%) had combined FVIII and FV deficiency. 228 (41.2%) patients had severe hemophilia. The most common complications were Epistaxis (n=59), Hemarthrosis (n=51) and Hemophilic Arthropathy (n=49).None of the patients were human immunodeficiency virus positive but 125 (22.6%) were hepatitis C virus positive and 2 (0.4%) were hepatitis B positive. Replacement therapy primarily relied on Cryoprecipitate and Fresh Fibrinogen. Plasma. Partial thromboplastin time (PTT) inhibitor was not arising. Conclusion. Most of the hemophilic patients have the severe type of the disease, this differs from that obtained by other studies elsewhere and it may be due to some degree of under diagnosis of the less severe forms of hemophilia. Implement a program of prophylaxis for hemo- philic arthropathy in children with severe hemophilia could be helpful. A more stringent policy for blood product usage, HCV screening and HBV vaccination is needed to abolish these diseases in patients with hemophilia.

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DISSEMINATED INTRAVASCULAR COAGULATION IN AN ANGIOMYELOLASTIC T-CELL LYMPHOMA

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Introduction. Disseminated intravascular coagulation (DIC) is a syndrome suggested by clinical signs and laboratory tests. The diagnosis may be based on the new ISTH overt-DIC score or other parameters including soluble fibrin monomer complexes (SFMC), antithrombin and von Willebrand factor (vWF) Consumption. Angiomyelolastic T-cell lymphoma (ATL) is an uncommon lymphoma. We report the case of a 71-year-old woman who presented ATL with an inaugural DIC associated with additional adverse prognosis parameters like SFMC, antithrombin III (AT) and protein C levels. Clinical observation. This woman was admitted to the hospital for severe health alteration. Clinical examination showed pallor, diffuse enlarged peripheral lymph nodes, hepato-splenomegaly, purpuric vasculitis and bruising. Laboratory analyses showed (→ rad) of DIC: hemoglobin 9→7g/dL (nr: 12-16), platelets 64→39x1012/L (nr: 150-400), lactic dehydrogenase (LDH) 1272 U/L (nr: 240-480), AT was 42%, 34% (nr: 80-120), D-dimers were up to 12,95→9g/mL (normal<0,5). STA-liestat FM, a new immuno-turbidimetric method of fibrin monon, was positive during the time of DIC, up to 17 µg/mL (normal<6) while F-test was positive once. Autoimmune features included severe not-HIV CD4 lymphopenia (80/mm³), positive Coombs test and polycythaemia (41g/L). Chest and abdominal CT-scan showed diffuse adenomegaly, hepatosplenomegaly and ascites. A lymph node biopsy showed diffuse infiltration by CD3+, CD4+, CD20, CD10- T-cells. Cytometry revealed T- and B- activation with 73% HLA- DR+, 15% CD25+ B, 11% CD85+ + B, and 8% of plasma cells. FCR was positive only on a paraffin tissue section. Angiomyelolastic T-cell lymphoma Ann Arbor stage IV with DIC was made. An oral chemotherapy (methylprednisolone 1 mg/kg/day and cyclophosphamide 50mg/day) was given. Two weeks later, DIC resolved with negative of F-test. Two months later, the patient was in complete clin-
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DERANGEMENT OF HEMOSTATIC PROTEINS IN HCV CIRRHOTIC PATIENTS: RELEVANCE TO HEMORRHAGIC DIATHESIS AND THROMBOTIC EPISODES
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Backgrounds. An altered coagulation profile resulting in decreased natural anticoagulant levels leading to haemorrhagic activation is described in patients with liver cirrhosis. The protein C system, a major physiologic regulator of haemostatic balance, controls thrombin production and guards against thrombotic episodes. Aims. This study was designed to assess the components of protein C system in HCV cirrhotic patients to determine whether alterations in these haemostatic proteins are related to degree of hepatic dysfunction and/or haemostatic activation and development of hemorrhagic diathesis or thrombotic episodes. Methods. Components of protein C (PC) system were assessed in 44 patients with different cirrhotic groups compared to controls (15 healthy subjects). Components of protein C system were assessed in 44 patients with different cirrhotic groups compared to controls (15 healthy subjects).

Results. Stimulation of the inflammatory process (increased TNF-α, NE and C4b-BP), endothelial injury (elevated TAT and d-imer) and increased consumption (prolongation of prothrombin time, platelet count and PC deficient) were measured in plasma by ELISA. Fibrinogen, functional activities of PC (PC Fh), plasminogen activator inhibitor-1 (PAI-1) and C4b-binding protein (C4b-BP) concentrations were also assessed. Results. The biology of Angiogenic factors in adult ALL appears different from children's ALL. The biology of Angiogenic factors in adult ALL appears different from children's ALL.

Conclusions. The study suggests that NE and TNF-α contribute to haemostatic alterations in patients with viral hepatitis C liver cirrhosis, and emphasizes the clinical significance of protein C as a sensitive parameter for hepatic dysfunction and protein S and PAI-1 as reliable prethrombotic markers in these patients.

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SCREENING TEST FOR VON WILLEBRAND DISEASE IN CHILDREN: A PFA-100 CLOSURE TIME
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Von Willebrand disease (VWD) is the most common inherited bleeding disorder so clinical symptoms, positive family history, and good sensitive laboratory assays should be used for diagnosis. Definitive diagnosis of type 1 von WWD remains undiagnosed because of mild symptoms and borderline laboratory values. We evaluated the sensitivity of closure time (CT) of the PFA-100® system with both cartridges (collagen/epinephrine (EPI) and collagen/ADP (ADP)). Over a 5 year period (2000 - 2006) testing was performed on blood samples from 44 patients (age 0.3 - 19 years, medi-age 12 years; 18 males and 26 females) registered in the Center for haemophilia and other bleeding disorders at the Children's hospital in Medical centre Ljubljana, Slovenia. In house reference ranges for children population were previously established and are 78 - 160s for EPI test and 55 - 124s for ADP CT. We found that all 6 patients with type 2 or 3 VWD had prolonged CT with both EPI and ADP cartridges. Among 38 patients with definite type 1 VWD 31 patients had prolonged CT with either of cartridges. The sensitivity of test for V1 VWD was 82%, 76% of them (76%) had prolonged CT with EPI cartridge and 23 (59%) with ADP cartridge, and ADP cartridge. On the day of CT testing 7 of 38 patients (18%) with type 1 VWD had results in normal range with both cartridges. Only 3 of these patients had low VWF level, other 4 (10%) had normal VWF level. Sensitivity of the PFA-100® system established in our patients with type 1, 2 and 3 VWD was 84%. When clinical suspicion is strong, testing for CT and VWF level should be repeated in spite of normal CTs and normal VWF level.

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ANGIOGENIC FACTORS PATTERN IN LYMPHOCYTIC LEUKEMIA
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Backgrounds. Angiogenesis is a crucial event in development and progression of solid tumors. Although the role of angiogenesis and angio-genic status is well studied in acute myeloid leukemia, its role in lymphocytic leukemia remains insufficiently characterized. Aim. is to investigate the profile of the systemic components of angiogenic factors in patients with lymphoblastic leukaemia at diagnosis (n=12), in remission (n=14), and chronic lymphocytic leukaemia at diagnosis (n=15), in remission (n=9) in order to determine their clinical validity. Methods. By ELISA technique, we assessed the serum vascular endothelial growth factor (VEGF), tumor necrosis factor-alpha (TNF-α), endothasin and basic fibroblast growth factor (bFGF) levels in culture supernatants of peripheral blood mononuclear cells collected from ALL and CLL patients at diagnosis and in remission. On the other hand serum matrix metalloproteinase-9 (MMP-9) was assayed in remission only. Results. In ALL patients, sVEGF were significantly lower than control (p<0.001) and increased near control levels in remission (p=0.05). In contrast, bFGF level was significantly higher than that in control (p=0.05) and decreased near control level in remission (p=0.05). Both serum TNF-α and endo-statin levels showed no significant difference both at diagnosis (p=0.05) and in remission (p>0.05) comparing to control level. Serum MMP-9 level was significantly lower than that in control (p=0.004). In CLL patients, serum VEGF, TNF-α and bFGF levels in culture supernatant were significantly higher than control (p=0.001; p=0.009; p=0.002 respectively) and decreased near control level in remission (p=0.05 for All). Serum endo-statin levels showed no significant difference at diagnosis and in remission comparing to control level (p=0.05). Serum MMP-9 level at diagnosis was significantly higher than that in controls (p=0.009). In conclusion: The biology of Angiogenic factors in adult ALL appears different from CLL. Although the angiogenesis have a vital role in CLL its role in adult ALL is not so clear.

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ASSOCIATION OF PLASMINOGEN ACTIVATOR INHIBITOR-1 (PAI-1) GENE POLYMORPHISM AND CHANGES IN PAI-1 PLASMA CONCENTRATIONS WITH STROKE
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Background. Stroke is a major cause of morbidity and mortality, and rates as one of the leading causes of death and disability. As inhibitor of fibrinolysis, high levels of plasminogen activator inhibitor type 1 (PAI-1) gene polymorphism and changes in PAI-1 plasma concentrations with stroke. Methods. We performed a case-control study performed on 173 patients aged 32-84 years with first ischemic stroke, confirmed..
by CT. Exclusion criteria included non-atherosclerotic causes, and patients on oral anticoagulants. Controls (n=271) were age- and sex-matched, without a history of stroke. Genotyping was done by PCR-SSP (4G/5G) or PCR-RFLP using Xho I ((G/-844)A); PAI-1 and t-PA levels were assayed by ELISA. Results. Higher frequencies of the 5G allele (p=0.024; RR=1.72) and the -844 A/A genotype (p=0.032; OR=1.71; 95% CI=1.07-2.73) was seen in patients, while higher frequencies of the -844G allele (p=0.023; RR=0.584) and the 4G/4G genotype (p=0.03; OR=0.58; 95% CI=0.36-0.94) were found among control subjects. Complete linkage disequilibrium was seen between the 4G and -844G alleles, and between the 5G and -844A alleles in patients (p=0.022). While PAI-1 antigen levels were increased in 4G/4G, more than -844 A/A carriers, and were associated with reduced t-PA levels, significant increases in PAI-1 levels were seen between cases and controls, irrespective of the genotype.

Summary/Conclusion. Whereas significant differences were seen in the distribution of some PAI-1 4G/4G and G-844A variants between cases and controls, yet their modest influence on PAI-1 levels and activity (t-PA) suggests the contribution of other stroke-associated factors in modulating PAI-1 levels and activity.

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**VASCULAR AND SINUSOIDAL ENDOTHELIAL ACTIVATION, PROLIFERATION, DIFFERENTIATION AND ERYTHROPHAGOCYTOSIS; ULTRASTRUCTURAL FINDINGS ON A CASE OF AUTOIMMUNE LYMPHOPROLIFERATIVE SYNDROME (ALPS)**

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**Backgrounds.** Since 1991, one of us (Sencer H) has reported that; vascular endothelial cells have reserved the capacity of stem cell and can activate, proliferate, and differentiate to other stromal and hematopoietic cells in health and diseases. Activated endothelial cells can migrate to the stroma or circulate in the vascular lumen as circulating progenitor endothelial cells (CPEC/CPEC) after plumping and detaching from basal lamina. Besides, viral replications and damages on erytocytes were clearly demonstrated ultrastructurally by Sencer H in 1995. Aims. The aim is to provide morphological basis of functional modifications occurring in the disease. This is the first ultrastructural study on ALPS, to our knowledge. Case report and Methods. The patient was healthy until the age of 6 months when he presented with disseminated vesicular skin lesions, generalized lymphadenopathy, hepatosplemégaly, tachycardia and was diagnosed with severe varicella zoster virus (VZV) infection. Coombs positive(IgG) hemolytic anemia, thrombocytopenia, elevated immunoglobulin levels and severe proteinuria were detected. CMV IgM and IgG were both found to be positive. At the age of 10-month CMV IgM and whole blood polimerase chain reactions analysis for CMV were negative. He presented with Evans syndrome symptoms and he was diagnosed as ALPS after the detection of increased percentage of double negative T cell population in the peripheral blood. The patient underwent splenectomy at the age of 20 months because of refractory thrombocytopenia. Material for this study were obtained during spleenectomy and performed EM preparation. Semi-thin sections were stained with toluidine blue-borax. Thin sections were contrasted with uranyl acetate/lead citrate and observed with JEOL100BEM.

Results. Red pulp was widespread, but white pulp wasn’t distinctive with increased folicular hyperplasia and prominent marginal zone in the spleen. Increased fibrinotic elements some of which related several arteries in plane of sections and plasmacytes were seen. Virus-like particles were observed. Activation and proliferation of vascular and sinusoidal (littoral) endothelial cells had occurred. Some of them were committed to erythropagocytes which were became large and shuttle shape. Their cytoplasms were full with erytrocytes, erytrocyte fragments and/or phagoctytic end-products. Erytrocytes probably damaged with viruses- were internalized by endothelial cells, but could not been digest totally. Both of the activated and phagoctytic endothelial cells could detach from their original sites and move to the sinusoidal and/or vascular lumen. These could functionally be named circulating endothelial progenitor cells (CPEC) and circulating erythrophyagocytic endothelial cells (CEPEC) respectively. These were neither sinusoidal histiocytes, nor cordal macrophages classical. Conclusions. We suggest that the splenic endothelial cells have erythropagocyte activity in ALPS. Viral replication on erytrocytes and/or endothelium may be causative agent. Endothelium should be most important key system in the health and disease. Electron microscopy is useful to avoid misinterpretation.

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**ENDOTHELIAL MICROPARTICLES AND MARKERS OF COPPER METABOLISM AS NOVEL INDICATORS OF ANGIogenesis IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA**

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**Backgrounds.** Angiogenesis is currently considered an important process in biology of B-cell chronic lymphocytic leukemia (B-CLL). Copper is an important cofactor for some angiogenic factors. Elevated serum levels of copper (Cu) and its transport protein coeruleoplasmin (CP) have been reported in patients with advanced cancers. Endothelial microparticles (EMPs) are fragments of endothelial cells which are produced during endothelial proliferation or damage and circulate in peripheral blood. Neither parameters of copper metabolism nor EMPs have been used so far to assess angiogenesis in B-CLL. Aims. To analyze serum concentrations of Cu and CP and quantify EMPs in patients with B-CLL. Methods. We measured serum Cu and CP in 19 patients with B-CLL diagnosed according to NCI-WG criteria. Cu was measured using chromatography and CP by immuno turbidimetry. EMPs were analyzed in 20 B-CLL patients and 10 healthy donors using two-colour flow cytometry of platelet-poor plasma. CD105 (endoglin) and CD144 (VE-cadherin). CD41 was used as a platelet marker. Results. Cu and CP were detectable in all B-CLL patients. Both markers were in normal range (Cu: mean ± SD [standard deviation], 18.13±3.98 µmol/l, 95% CI [confidence interval] of mean, 16.21-20.05 µmol/l; CP: mean±2SD, 0.294±0.62 g/l, 95% CI of mean, 0.264-0.324 g/l). Neither Cu nor CP were significantly different between B-CLL patients with stable (n=7) and progressive (n=12) disease (p=0.77 and 0.54, respectively). There was a statistically significant increase of CD144+105+ microparticles (mean±SD 142.8±22.4 µL, 95% CI of mean, 95.8-189.7/µL) in B-CLL patients when compared to control group (mean±SD [standard deviation], 60.8±2.4 ± 22.4/µL, 95% CI of mean, 57.6-84.0/µL; p=0.008). There was no significant difference between patients with. Conclusions. Our study is the first to one report measurement of endothelial microparticles and markers of copper metabolism as angiogenesis indicators in CLL. However, neither serum Cu nor CP were significantly elevated in B-CLL patients over controls. In addition, we did not observe differences in Cu or CP levels between patients with stable vs progressive disease. Furthermore, we found elevated numbers of CD41+105+ (aggregates of platelets and EMPs) but not CD144+ or CD105+ EMPs in B-CLL patients. Larger study is clearly warranted to confirm these findings and perform a detailed statistical analysis including comparison with other angiogenic markers.

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Elimination of iron in hereditary haemochromatosis patients treated with erythrocytapheresis

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Backgrounds. Hereditary haemochromatosis (HH) is an inherited, autosomal recessive disorder of iron metabolism that causes the body to absorb and store an excess amount of iron resulting in the progressive accumulation of iron in the liver, pancreas, heart, joints, and pituitary gland leading to potentially serious complications including cirrhosis of the liver, diabetes, and heart problems. The effective treatment is the regular whole blood removal which causes erythropoiesis activation and leads to decrease of iron stores. Red cell apheresis is an optional method for removing higher amounts of erythrocytes in one session. The aim of this study was to evaluate the effectiveness of erythrocytapheresis in the treatment of HH. Methods. Repeated erythrocytapheresis were performed in 17 patients (15 x C282Y homozygotes, 2 x C282Y + H63D heterozygotes) using Haemonetics MCS 3P cell separator (protocol TAE) in which red cells were removed from patients in 2–5 cycles; plasma and buffy-coat were reinfused. Collection time, donor convenience, side effects and red cell yield were recorded and analysed. Samples for hematology and iron studies in patients were drawn, analysed and compared to baseline levels. Results. 376 (3–70) red cell apheresis in 17 patients (15 male, 4 female), age 49.9 (32–67), height 175.7 cm (160–190), weight 82.5 kg (55–110), TBV 5186 mL (3627–6501). Procedure time was 32–87 min. Mean Hb level decreased from 141.7 g/L (115–185) before the procedure to 121.6 g/L (83–130). Ferritin values decreased from 1199 ng/mL (268–3998) to less than 25 ng/mL (7–23) in each of patients. The drop in ferritin level was 175 ng/mL (67–358) per month and 86 ng/mL (41–135) per one apheresis, respectively. Conclusions. Procedures were well tolerated by patients, no serious side effects were seen, 21 mild citrate reactions (7.6%) were noted. Red cell apheresis is an effective procedure of iron stores reduction in patients with the hereditary haemochromatosis. Decrease of iron stores in patients is individual and depends on many factors.

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THERAPEUTIC LEUKAHERAPESIS EXPERIENCE OF A SINGLE CENTRE

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Therapeutic Leukapheresis (TL) is an option in the management of patients with hyperleucocytosis, especially associated with leukostatic symptoms. Nevertheless, its clinical and analytical benefit is not well documented in the literature. The aim of this study was to retrospectively analyse the TL performed in our Centre, between January 1998 and December 2005 and also to evaluate its efficacy and complications. During this period, 28 TL were performed in 15 patients (9 men/6 women), with a median age of 22 years (range 2-78), diagnosed with Acute Lymphoblastic Leukaemia (n=6), Acute Myeloblastic Leukaemia (n=7) and Chronic Myeloid Leukaemia (n=5). Most of the patients (n=14) initiated TL within one week after the diagnosis. One pediatric patient with CML and an initial white blood cells (WBC) count of 300000/μL did not have leukostatic symptoms. The other presented cerebral (lethargy, aphasia, dysartha, altered vision, intracranial haemorrhage) and/or pulmonary (dry cough, respiratory distress and alveolar haemorrhage) manifestations. Each patient was treated with a median of 2 TL (1-4). Apheresises were performed in a Cobe Spectra cell separator in the Intensive Care Unit. The mononuclear cells program (MNC) was selected in 20 procedures and the polymorphonuclear cells program (PMN) in the other 8 cases. A median of 3 blood volumes per TL was processed (1-4). An efficacy index (Ei) was calculated in order to monitor the procedures: Ei = (total collected WBC / total pre-apheresis patient WBC) x 100. The median pre-apheresis WBC count was 213×10^3/L (65-856), which had a corresponding median leukocit of 8 ml/dL (2-26). The median Ei of all TL was 20% (0-47) and when considering each procedure, the PMN had a median of 23% (16-47) and the MNC achieved 18% (0-80). The median WBC count, 1 hour and 24 hours after TL, was 17×10^3/L (45-650) and 84×10^3/L (09-491), respectively. Serious complications occurred in 4 patients leading to TL interruption. Those were: respiratory arrest, hypertension, respiratory failure and mucocutaneous haemorrhage; however no deaths occurred. Hypocalcaemia related side effects were observed in 15 patients, but promptly reverted with calcium gluconate administration. After TL, clinical improvement was observed in patients who survived at least for one month. The remaining 9 patients, maintained or worsened their condition and all had an early death (<30 days). The overall survival rate at 6 months after TL was 40%. In summary, in this Centre, the majority of patients who underwent TL were clinically ill. Even though, the survival rate was similar to the reported in the literature. The lack of immediate clinical improvement can be a sign of a poor prognosis. The PMN program was found to be more effective than the MNC and the EI revealed an easily calculated and reliable indicator. Conclusions were limited due to the reduced number of patients in the study. It is important to find standard indicators to technically and clinically monitor the TL, in order to allow multicentric comparisons from the data available.

RETROSPETIVE ASSESSMENT OF THE GLOBAL QUALITY OF LIFE OF PATIENTS WITH ACUTE MYELOID LEUKAEMIA AFTER HSCT FROM NURSES PERSPECTIVES: FINDING FROM A CROSS-SECTIONAL AND RETROSPECTIVE STUDY

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Backgrounds. The cross-sectional and retrospective study analyses the selected factors which influence global quality of life (QoL) of patients with acute myeloid leukaemia (AML) after the hematopoietic stem cell transplantation (HSCT). Aims. To verify the applicability of the Czech version of an international generic European Quality of Life Questionnaire - Version EQ-5D with the evaluation of global QoL in patients with acute myeloid leukaemia (AML) after HSCT at the 1st and 2nd Clinical Hematology of the 2nd Internal Clinic in the University Hospital and Medical Faculty of Charles University in Hradec Kralove Czech Republic and to evaluate the global QoL in patients with AML after HSCT at the Department of Clinical Hematology of the 2nd Internal Clinic in the University Hospital and Medical Faculty of Charles University in Hradec Kralove, Czech Republic. Methods. The total number of respondents after the transplantation from 2001 to 2003 was 19 and the return rate of questionnaires was 63% (12 respondents: 9 respondents with AML after autologous HSCT, 3 respondents with AML after allogeneic HSCT. The mean age of patients with AML was 47.3 years old (range 27-68) and the male / female ratio was 1:7/3. The Czech version of a international generic EuroQoL Questionnaire - Version EQ-5D was used. The influence of monitored factors (age, sex, education, marital status, polymorbidity, nicotinism, religion, type of HSCT and the time lapse from the HSCT) on global quality of life of patients was determined by means of dispersion analysis. The above mentioned factors proved statistically significant dependence of EQ-5D score and EQ-5DVAS on age (in both cases p<0.01), education (in both cases p<0.05) and polymorbidity (in both cases p<0.05). Conclusion: EQ-5D score (dimensions of EQoL) and EQ-5D-VAS (a subjective health condition) significantly decrease with increasing age, religion, nicotinism, education and polymorbidity on patients with AML after HSCT. The global QoL of patients with AML after HSCT is high (mean EQ-5D score 75.1%, mean EQ-5D-VAS 67.5%).

A 5+5 YEAR EUROPEAN NON-INTERVENTIONAL SAFETY STUDY COMPARING ANAGRELIDE HYDROCHLORIDE (AGRIQ) WITH OTHER CYTOSCRIPTIVE TREATMENTS IN AT-RISK ESSENTIAL THROMBOCYTHEMIA SUBJECTS

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Backgrounds. Long-term data supporting the use of the cytoreductive agents in the management of elevated platelet counts remains sparse, particularly when analysing long-term safety. Anagrelide is a selective, non-cytotoxic platelet reducing agent that has been used extensively...
The goal of the treatment was to lower the platelet count below 500 × 10^9/L. B. Interferon was administered at a dose of 3 MU, subcutaneously, three times weekly. In Group A, 24 patients received anagrelide and up to 3000 at-risk ET subjects receiving other cytoreductive therapies. All cytoreductive agents must be prescribed in accordance with the appropriate product information. Subjects may be newly diagnosed or continuing their existing medication for the treatment of ET. Concomitant medication use is at the discretion of the investigator. Data will be collected without any interference with the treatment choice of physicians and will be captured electronically by use of a web-based registry utilizing electronic case report forms. An initial 5-year study period will focus on the collection of data related to a number of pre-defined events. These include complications of the disease (thromboembolic and hemorrhagic events as well as transformation) and possible toxic complications (congestive cardiac failure, cardiomyopathy, severe mucocutaneous disorders, pulmonary hypertension, pulmonary fibrosis/interstitial pneumonia, pancreatitis, rhadomyolysis/myalgia and non-haematological malignancy). Death, as well as the incidence of serious adverse events related to current ET therapy will be recorded. Events will be evaluated by an independent Event Validation Panel. If required, based on review of data from the initial 5-year phase, data will be collected during a second 5-year study period to assess selected pre-defined events including pregnancy (and progeny) outcomes, serious adverse events related to the current ET therapy, and other events as defined by the steering committee. Results. Study recruitment began on 30 May 2005. To date, 364 subjects have been recruited on target with a median age of 41 years (range 14-70). Group A included 15 males and 22 females, with a median age of 42.5 years (range 22-74). Group B included 15 males and 22 females with a median age of 41 years (range 14-70). At diagnosis, 4 patients in Group A presented with thrombotic episodes vs 3 patients with thrombotic and 1 with hemorrhagic episode in Group B. If required, based on review of data from the initial 5-year phase, data will be collected during a second 5-year study period to assess selected pre-defined events including pregnancy (and progeny) outcomes, serious adverse events related to the current ET therapy, and other events as defined by the steering committee. Results. Study recruitment began on 30 May 2005. To date, 364 subjects have been recruited on target with a median age of 41 years (range 14-70). Group A included 15 males and 22 females, with a median age of 42.5 years (range 22-74). Group B included 15 males and 22 females with a median age of 41 years (range 14-70). At diagnosis, 4 patients in Group A presented with thrombotic episodes vs 3 patients with thrombotic and 1 with hemorrhagic episode in Group B. Interferon was administered at a dose of 3 MU, subcutaneously, three times per week and atagrelide at a dose range of 1-3 mg per day. The goal of the treatment was to lower the platelet count below 500 × 10^9/L. Results. All patients achieved the proposed goals of treatment. One thrombotic or hemorrhagic complication in Group A vs 6 in Group B were recorded during therapy. These complications were: in Group A an hemorrhagic cerebral episode and in Group B heart attack and mesenteric vein thrombosis in the same patient, femoral vein thrombosis, transient ischemic cerebral episode, erythromelalgia and severe nasal hemorrhage. None of these episodes were fatal and all patients recovered. The median follow up was 68.5 months (range 4-196) under interferon-α and 32 months (range 1-94) under anagrelide. In Group A we did not observe any thrombotic event, while we recorded one hemorrhagic event for a rate of 0.6% per year after 155 persons-years of follow up. In Group B we observed hemorrhagic events in patients with ET during therapy with interferon-α or anagrelide. Despite the fact that this is a retrospective, no randomised study with a small number of patients, these results made us more sceptical regarding the use of anagrelide in patients with ET, as a first-line treatment.
Systemic mastocytosis. An Italian multicentric retrospective survey


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Background/aims. To evaluate clinical and molecular features, and outcome of patients (pts) with Systemic Mastocytosis (SM). Methods. A retrospective revision of 26 cases of SM, diagnosed in 11 Italian Hematology Divisions between 1995 and 2006. Results. 26 new cases of SM were collected and classified according to the WHO criteria: Mast Cell Leukemia in 12 pts, Aggressive SM in 10 and Indolent SM in 3; the remaining one had SM with associated clonal non-mast-cell lineage hematologic disease (AML). Skin was the principal extramural organ involved by uncontrolled proliferations of MC (17 pts) followed by spleen (13), liver (12), and cardiovascular system (12). In 61% of cases constitutional symptoms (fatigue, itchiness and abdominal pain) were present. Molecular biology studies were performed in 10 pts: 12 showed the c-kit point mutation D816V, in 3 pts additional gene defects and karyotype abnormalities were recognized. Treatments were very heterogeneous, and the same patient could have received different therapies after failure of the previous one. Seven patients were not initially treated: 5 maintained a stable disease, while 2 had a progressive clinical course. Interferon-α (404 μg/m²/day) was used in 10 pts (10 as first line therapy, 4 and 1 as second and third line respectively); c-kit mutation was present in 9 of these 15 pts. A partial response was obtained in only one of them (response rate 11%); among the remaining 6 patients without the c-kit mutation, partial or complete remissions were obtained in 2 and 1 pts respectively (53% and 17%). Interferon-α (500 million units s.c. weekly) was employed in 6 patients (3 as first line therapy, 2 as second and 1 as third line): a partial remission was achieved in one case only (17%). 2 CDA (0.14 mg/kg) was administered in 3 pts (1 as first, 1 as second and 1 as third line therapy) registering a partial remission in all of them. One patient performed only radiotherapy and achieved a pure clinical major response. In 4 cases were used other chemotherapies (in 2 pts as first and in other 2 as second therapy) with no response, and in 1 case chemotherapy received steroid therapy, not in association with other drugs, obtaining in 1 case a partial response. Two patients underwent stem cell transplantation as second and fourth line respectively, obtaining both a complete remission. Two pts (8%) who had received conventional chemotherapy only, died for mastocytosis; a third patient in complete remission of disease died for accidental causes. The 10-years survival rate is about 88%. Summary/Conclusions. Our results suggest that SM is a very rare disease, but although severe and life-threatening mediator-related symptoms, the mortality is low. D816V c-kit mutation is associated with relative resistance against imatinib. Among purine analogues, 2-CDA has shown interesting clinical major response, while IFN has not offered any benefit although the similarity between SM and myeloproliferative diseases. Because of the rarity of this disease, an effective standard of care is lacking: for this reason more data are needed to find new and successful therapeutic strategies, such as other tyrosine kinase inhibitors.

1106
Comparison of the results for the JAK2 V617F mutation detection by two methods: allele specific PCR and restriction digestion assay on polycythemia vera and essential thrombocythemia DNA samples

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JAK2 V617F is a clonal acquired mutation found in the majority of patients with polycythemia vera (PV), and a significant number of patients with essential thrombocythemia (ET) and chronic idiopathic myelofibrosis (CIMP). The incidence of this mutation ranging from 65% to 97% in PRV patients, from 25% to 57% in ET patients and about 50% in MMM depending on the study. These variations in percentage of patients involved is likely due to the criteria used for diagnosis and also the sensitivity of the assay used to detect this mutation. Allele specific PCR and restriction digestion assay by enzyme Ba XI are usable techniques for its detection. The incidence of JAK2 V617F mutation was compared to that published in the literature. The diagnosis of ET and PV was established followed World Health Organization classification. 19 patients with PRV and 42 patients with ET were included in the study. EDTA peripheral blood was drawn and used for the isolation of the granulocytes by ficoll density centrifugation followed by dextran sedimentation. DNA was isolated from granulocytes by High Pure PCR Template Reagent kit from Roche The allele specific PCR was carried out as described in Baxter EJ et al (Lancet 2005; 365:1054-61). Restriction digestion assay followed by agarose gel electrophoresis by ethidium bromide staining. The concordance between these two methods was 100%. The percentage of positivity for JAK2 V617F mutation on DNA samples from granulocytes from peripheral blood in ET and PV patients. In our hands the sensitivity of this two methods was the same. The percentage of positivity for JAK2 V617F mutation in ET and PV patients was similar of that published in the literature and was in the upper part of the range.

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Microvessels density (MVD) and vascular endothelial growth factor (VEGF) immunohistochemical expression in Ph(+) chronic myeloproliferative disorders (CMPDs)

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There is increasing evidence that neovascularization may play an important role in haematological malignancies and in particular in lymphomas, acute leukaemias and myelodysplastic syndromes. However, few studies have been performed in order to evaluate this phenomenon in Ph(+) CMPDs. Increased angiogenesis in chronic idiopathic myelofibrosis (CIMP) and high serum concentration levels of VEGF, the most potent direct-acting angiogenic factor, in CMPDs were reported. Recently, an increased immunohistochemical expression of VEGF was demonstrated in CMPDs. A new classification of chronic CMPDs was worked out by the WHO, which highlighted the importance of bone marrow biopsy (BMB) in differential diagnosis and in the evaluation of myelofibrosis. In addition to standard therapy, new therapeutic ways of approaching and directly targeting endothelial cells or VEGF have been experimented in CMPDs, with variable results. The aim of this research was to examine the MVD and VEGF immunohistochemical expression in the different categories of Ph(+) CMPDs, according to the new WHO classification. We examined the BMBs of 90 CMPDs patients, classified according to the WHO classification. In particular, there were 30 cases of essential thrombocythaemia (ET), 30 of CIMP (10 CIMP-0, 10 CIMP-1 and 10 CIMP-2+3) and 9 cases of Ph(+) CMPDs - Haematologica 2005) and 30 of polycythaemia vera (PV) (20 polycythaemic phase and 10 polycythaemic myelofibrosis). We analyzed 20 non-pathologic BMBs as normal controls. MVD analysis was performed according to the hot-spot method, using an anti-CD34 antibody. The VEGF immunohistochemical expression was expressed as VEGF index, according to the mathematical formula [VEGF(i) = VEGF(+) x BMB cellularity/100]. All statistical tests were performed at the 5% significance level (p<0.05) (Anova oneway). Hot-spot MVD and VEGF(i) immunohistochemical results are described in Table 1.

Table 1. Hot-spot MVD and VEGF(i) immunohistochemical results.

<table>
<thead>
<tr>
<th>Vessels Medium</th>
<th>N. ± SD (range)</th>
<th>VEGF(i) ± SD (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>7.5±3.6 (4.6-16.3)</td>
<td>0.08±0.009 (0.01-0.15)</td>
</tr>
<tr>
<td>ET</td>
<td>10.1±4.4 (3.3-19.6)</td>
<td>0.12±0.05 (0.05-0.28)</td>
</tr>
<tr>
<td>CIMF-0</td>
<td>21.5±3.8 (15-26)</td>
<td>0.20±0.09 (0.12-0.42)</td>
</tr>
<tr>
<td>CIMF-1</td>
<td>29.3±5.4 (17.6-38.3)</td>
<td>0.36±0.18 (0.12-0.64)</td>
</tr>
<tr>
<td>CIMF 2+3</td>
<td>27.3±6.3 (20-44.6)</td>
<td>0.26±0.12 (0.01-0.42)</td>
</tr>
<tr>
<td>PV</td>
<td>17.3±7.4 (5.6-33.6)</td>
<td>0.21±0.15 (0.09-0.66)</td>
</tr>
<tr>
<td>MF post-PV</td>
<td>31.9±7.3 (15-40)</td>
<td>0.49±0.19 (0.05-0.64)</td>
</tr>
</tbody>
</table>

There is no difference in MVD and VEGF(i) expression between ET and control group. Moreover MDV and VEGF(i) proved to be much
higher in CIMF and PV than in the control group. MVD and VEGF(1) in fibrotic CIMF (CIMF-2-3) have been demonstrated statistically different from MDV and VEGF in myelofibrosis post-PV. Our analysis identified significant biological differences between the various types of myelofibrosis and could serve as a rationale guide in the antiangiogenic therapy.

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**JAK2V617F, PRV-1 EXPRESSION AND ENDOGENOUS ERYTHROID COLONIES GROWTH IN PATIENTS WITH POLYCYTHEMIA VERA**


**Background.** Polycythemia vera (PV) is a chronic myeloproliferative disorder (MPD) characterized by a primary increase of red cell mass. One of the WHO diagnostic criteria for PV is the in vitro endogenous erythroid colonies (EEC) formation. Some molecular alterations have been associated to MPD, and currently the most indicative molecular markers are the overexpression of PRV-1 and the genomic mutation JAK2V617F. Both these alterations have been found in the majority of PV cases and in particular JAK2 has been associated with the ability to form EECs, and seems to have a causal or a strongly contributory role in the pathogenesis of the MPDs and in particular of this primary erythrocytosis. Furthermore it has been suggested an allele dose-dependent association that links JAK2V617F and expression of PRV-1. **Aims.** To evaluate the association between EEC formation and the molecular alterations considered, we analyzed 21 cases of PV for EECs, JAK2V617F and PRV-1 expression. **Methods.** JAK2V617F was performed by allele-specific amplification; PRV-1 expression was normalized on GAPDH expression, analyzed with the ΔCt method and expressed as relative quantification (RQ); EECs were detected on methylcellulose-based medium with and without erythropoietin addition. **Results.** JAK2V617F was found in 17/21 (81%), and 4 of the JAK2V617F-positive cases presented only the mutated allele. All the patients analyzed showed EEC growth. PRV-1 expression was evaluated in 14 patients at the diagnosis and in 7 patients under hydroxyurea administration; overexpression resulted in 15/15 and 3/7 (43%) patients, respectively. The RQ mean in the JAK2V617F-negative, heterozygous JAK2V617F-positive and homozygous JAK2V617F-positive groups of untreated patients resulted 3.7 (1.2-10.4), 10.95 (3.6-25) and 12 (1.2-24), respectively. A control group of 7 patients with secondary erythrocytosis was also analyzed and resulted negative for JAK2V617F and PRV-1 expression. **Conclusions.** We did not find any association between JAK2V617F and EEC growth, since the RQ was similar in JAK2V617F-positive and JAK2V617F-negative patients. Furthermore, we did not find any difference between cases analyzed at the diagnosis and cases that were in therapy. Considering hematological parameters such as white blood cells and platelet counts, and hemoglobin level, the subgroup JAK2V617F-positive did not show higher values than JAK2V617F-negative active patients. Thus we did not find distinctive hematologic characteristics that differentiate EEC JAK2V617F-positive or negative.

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**JAK2V617F MUTATION, PRV-1 OVEREXPRESSION AND EECs IN PATIENTS WITH ESSENTIAL THROMBOCYTHENIA**

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**Background.** JAK2V617F is a genomic mutation associated to myeloproliferative disorders such as polycythemia vera (PV), essential thrombocythemia (ET) and idiopathic myelofibrosis, that has been highly correlated with the ability to form endogenous erythroid colonies (EEC) and with PRV-1 overexpression. Nevertheless JAK2V617F mutation and PRV-1 overexpression are present in the majority of PV but in a percentage significantly lower of patients with ET, suggesting a different role or effect of these molecular alterations in the different chronic myeloproliferative disorders. In particular the presence of JAK2V617F mutation is associated in ET patients to multiple clinical features resembling PV, suggesting the need of a new diagnostic classification based on the genotypic profiles. **Aims.** In the attempt to evaluate the incidence of the genetic alterations described, and the association to hematological features, we analyzed JAK2V617F, PRV-1 expression and EEC growth in a cohort of 46 patients with essential thrombocythemia. **Methods.** JAK2V617F was performed by allele-specific amplification; PRV-1 expression was normalized on GAPDH expression and analyzed with the ΔCt method, the EECs were detected on methylcellulose-based medium with and without erythropoietin. **Results.** JAK2V617F mutation was present in 11/46 (24%) cases; PRV-1 was overexpressed in 12/43 (27.9%) cases, in particular 7/27 untreated patients (25.9%) and 5/16 (31.3%) patients in therapy; 30/39 (76.9%) cases showed the ability to form EEC. The statistical evaluation of the data showed a significant correlation between JAK2V617F and EEC growth (p=0.04, R=0.33), but the correlation between JAK2V617F and PRV-1 overexpression was not significant. **Conclusions.** Our study seems to be in accordance with previous reports regarding the incidence of the molecular alterations found, but the correlation was statistically significant only between JAK2V617F and EEC. In fact, considering PRV-1 overexpression, neither correlation was statistically significant, nor was found any allele dose-depending effect on PRV-1 expression by JAK2V617F mutation. Finally, we did not find a difference between cases analyzed at the diagnosis and cases that were in therapy. Considering hematological parameters such as white blood cells and platelet counts, and hemoglobin level, the subgroup JAK2V617F-positive did not show higher values than JAK2V617F-negative active patients. Thus we did not find distinctive hematologic characteristics that differentiate EEC JAK2V617F-positive or negative.

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**INCREASED ANGIOGENESIS IN CHRONIC IDIOPATHIC MYELOFIBROSIS: VEGF AS KEY ANGIogenic FACTOR**

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**Backgrounds.** Recent studies suggest that increased angiogenesis is implicated in the pathogenesis of chronic idiopathic myelofibrosis (CIMF). Its impact on prognosis, however, is still a matter of debate. Vascular Endothelial Growth Factor (VEGF) is a potent stimulator of angiogenesis which is expressed in virtually all types of malignancies. We therefore hypothesized that VEGF may also play a role as an angiogenic mediator in CIMF. **Aims.** The purpose of this study was to assess the prognostic value of bone marrow angiogenesis and its correlation with clinical parameters and cytogenetics in patients with newly diagnosed, untreated CIMF. Moreover, we aimed to investigate the expression of VEGF in bone marrow of CIMF and healthy controls. **Methods.** We included all patients who were diagnosed as having CIMF at our center and for whom adequate bone marrow sections and clinical data were available, who were deemed eligible. Each case was re-classified according to WHO criteria. As a surrogate marker for angiogenesis we used microvessel density (MVD) as assessed by CD34 staining on paraffin-embedded trephine biopsy specimens. VEGF expression was examined by standard
immunohistochemical technique. The cytogenetic phenotype was determined by FISH on de-paraffinized bone-marrow sections. Appropriate summary statistics were used for comparisons between groups; survival was calculated using Kaplan-Meier estimates. Parameters found to be of prognostic significance in univariable analysis were verified in a multivariate Cox regression model. Results. Fifty-five patients were included in this retrospective single-center study. Clinical, cytogenetic and immunohistochemical data were available for all patients. With a median follow-up of 52.4 months (range 1 - 142 months), the median overall survival of the study cohort was 76.8 months. With a median MVD of 45 per 0.747 mm² field (range 6-96) CIMF patients displayed a significantly higher degree of bone marrow microvessel density than controls (median MVD=19, range 4-73, p=0.001). In fact, 85% of CIMF patients displayed an elevated MVD compared to normal controls. MVD was elevated significantly at all CIMF stages (p=0.001) with equal distribution between the various degrees of fibrosis (MF 0 - 3). Accordingly, VEGF expression was significantly higher in CIMF patients (median 1.4 cells per 0.747 mm² field; p=0.01) and correlated with MVD (p=0.001). However, we found no correlations of MVD or VEGF expression with cytogenetics and clinical outcome, respectively. Conclusions. Our study confirms that bone marrow angiogenesis is increased in CIMF. In parallel, we found significantly elevated VEGF expression suggesting VEGF signalling to play a pathogenetic role and representing a potential therapeutic target in CIMF.

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JAK2-V617F MUTATIONAL ANALYSIS IN PATIENTS WITH MYELOPROLIFERATIVE DISORDERS
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Methods. An acquired mutation in Janus kinase 2 (JAK2) gene (characterized by a valine-to-phenylalanine substitution at position 617 [V617F] in the JH2 domain) has been recently described in the majority of patients with myeloproliferative disorders (MPDs). This mutation is associated with constitutive phosphorylation of JAK2 and its downstream effectors as well as induction of erythropoietin hypersensitivity in cell lines. However, its precise role remains to be determined. The aim of this study was to estimate the prevalence of the JAK2-V617F mutation, as well as its clinical and laboratory findings in patients with MPDs carrying the mutation. Materials and Methods. One-hundred and forty-two patients (52.7% had at least one confirmed arterial and/or venous thrombotic episode either at diagnosis (mainly, as well 1-2 years ago) or during the follow-up period (mean: 38.8 months, range: 2-171). Genomic DNA was extracted from bone marrow aspirates or peripheral blood using standard protocol. The JAK2-V617F mutation was detected using both allele specific PCR and PCR-RFLP assay. Variables analyzed included age, gender, survival, thrombotic events, WBC, Ht, Hb, PLT, Epo, LDH, and the presence of splenomegaly, hepatomegaly and anticoagulation antibodies both at diagnosis and during the follow-up period. Statistical analysis was performed by the SPSS software. Results. One-hundred and three patients exhibited the JAK2-V617F mutational changes (53 of 59 with PRV, 84.6%; 68 of 94 with ET, 70.02%; 7 of 9 with IF). Interestingly, the patients carrying the JAK2-V617F mutation were older at diagnosis (61.3 ± 8.5, p=0.026), displayed lower Epo levels (8 vs 19.6, p=0.001), higher Ht (45.6 vs 42.1, p=0.018) and Hb values (15.1 ± 13.8, p=0.021), and presented more often with thrombotic events (35.9% vs 20.5%, p=0.079) and splenomegaly (32.05% vs 17.9%, p=0.097). In multivariate regression analysis, Epo levels and the presence of thrombotic events were independent variables correlated with the presence of mutation (p=0.004 and p=0.038, respectively). Moreover, 4 out of 5 patients who exhibited progression of the disease (5 with ET to IF and two with PRV to IF and AML, respectively) displayed the mutation, both before and after the deterioration of the disease. Conclusion. MPDs with JAK2-V617F mutation may prove to be a different disease entity than MPDs without JAK2-V617F mutation, with distinct clinical and laboratory findings.

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ROSAI-DORFMAN DISEASE (SHML) WITH NODAL AND MULTIPLE EXTRANODAL INVOLVEMENT COMPLICATED WITH AUTOIMMUNE HEMOLYTIC ANEMIA - A CASE REPORT
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Background. Rosai-Dorfman disease or Sinus Lymphoidosis with Massive Lymphadenopathy (SHML) described by Rosai and Dorfman in 1969, is a rare disorder (432 cases in the SHML registry) of unknown etiology. The lymph node involvement is non-malignant. It is characterized by a non-malignant proliferation of distinctive histiocytic/phagocytic cells within lymph node sinuses and lymphatics in extranodal sites (50%-40% of cases). Aims. Presentation of a patient with a severe form of SHML with nodal and multiple extranodal involvement in skin, upper respiratory tract, parotid gland, thymus, complicated with autoimmune hemolytic anemia. Methods. A gypsy male patient, one year old, was admitted with high fever, night sweats, anemia, and loss of weight, inspiratory stridor, massive painless cervical and submandibular lymphadenopathy, high triglycerides and ferritin, markedly decreased number of CD4+ and CD4+/CD8+ ratio, normal number of NK cells; serologic markers for EBV, HHV, CMV, HIV were negative; rheumatoid factor and antinuclear antibodies negative. Repeated lymph nodes and skin lesions biopsies were performed. Histopathological examination showed reactive lymphadenopathy; finally, the recognition of sinus histiocytosis and the hallmark of the SHML histiocyte, the lymphophagocytosis (emperipolesis), completed with immunohistologic investigation (histiocytes CD68+, S 100 protein+, CD1a-) confirmed the diagnosis. The treatment with prednisone 60 mg/m² for two months was efficient but the clinical and biological symptoms relapsed one month after. The interferon treatment was totally inefficient. Taking into account the severity of the disease, the treatment was continued with dexamethasone, etoposide and cyclophosphor, for 52 weeks (HLH-94 protocol). The response was very good, with complete recovery maintained 32 months after completing the treatment. Some peculiar features of the case are interesting: common manifestations with the hemophagocytic lymphohistiocytosis (HLH) -important hepatosplenomegaly, high triglycerides and ferritin levels, lymphophagocytosis; the autoimmune hemolytic anemia - recently cases of SHML associated with autoimmune lymphoproliferative syndrome (ALPS) have been described. The possibility that SHML represents an acquired disorder of apoptosis has raised a special interest.

Conclusions. Being an extremely rare disease, SHML was recognized very late, despite its characteristic histopathologic features. The particular severity of the presented case, with extensive involvement, progressive evolution, the life threatening complications (mediastinal syndrome, hemolytic anemia), imposed an intensive treatment.
**1113**

**REACTIVATION OF FETAL HEMOGLOBIN IN BONE MARROW DERIVED CD133+ CELLS: HEMATOPOIESIS IN VITRO**

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**Backgrounds.** Switching of fetal hemoglobin (Hb F) in the adults was controlled with mechanisms that are unclear. Understanding of mechanisms underlying this process can be useful for treatment of \( \beta \) globin disorders. **Aims.** We investigated probable synergistic effects of Transforming Growth Factor-\( \beta \) (TGF-\( \beta \)) and Stem cell factor (SCF) on fetal hemoglobin expression in hematopoiesis in vitro. **Methods.** The bone marrow was collected from normal person. Mononuclear cells were isolated using Immunomagnetic beads. Isolated cells have cultured for two weeks in IMDM with 30% FBS supplemented with EPO (erythropoietin) alone as control group, EPO+SCF (first group), EPO+IGF-\( \beta \) (second group) and EPO+SCF+TGF-\( \beta \) (third group). Then, RT-PCR and flow cytometry analysis were done for detection of \( \gamma \) globin and Hb F, respectively. Also, the Colony assay was accomplished. **Results.** Flow cytometry analysis showed occurrence of 96% Hb F positive cells in differentiated population in presence of SCF and TGF-\( \beta \). This percent was higher than other group. These results were confirmed by increase of \( \gamma \) globin expression detected by semiquantitative RT-PCR in comparison with control. The hematopoietic colony forming assay showed that hematopoietic progenitor cells have ability to forming colony the same as untreated cells. **Summary/Conclusions.** In conclusion, the cytokines or its derivatives that are used in this study can be a suitable candidate for treatment and investigation purposes instead of conventional drugs that can increase the Hb F.

**1114**

**HIGH-DOSE MELPHALAN WITH OR WITHOUT PALIFERMIN IN MULTIPLE MYELOMA: A SELF- CASE-CONTROL STUDY**

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Oral, oesophageal, and lower gastrointestinal tract mucositis is a common complication of high-dose chemotherapy conditioning regimens used with peripheral blood stem-cell transplantations (PBSCT). Severe grades of mucositis are associated with higher morbidity such as infections, need for parental nutrition, opioid analgesics and prolonged hospitalization. Moreover, oral mucositis is reported by patients as the worst and most memorable complication of their transplant experience. The KGF (keratinocyte growth factor) palifermin stimulates the growth, differentiation, migration and survival of epithelial cells. Palifermin is now approved in the EU to decrease the incidence, duration and severity of oral mucositis in patients with haematologic malignancies requiring autologous hematologic stem-cell transplanation. Prior to marketing approval, Palifermin was made available to patients in the EU through a conditional authorization. **Aims.** To evaluate the effect of Palifermin on oral mucositis given during the second autologous transplant in 5 patients who underwent a double PBSCT for multiple myeloma. Three multiple myeloma patients were scheduled to receive treatment with high-dose (HD) Melphalan (200 mg/m\(^2\)) followed by two successive autologous PBSCT. At the time of second autograft, patients received prophylactic intervention with intravenous Palifermin 60 \( \mu \)g/kg/day for 3 days before HD Melphalan and 3 days after PBSCT. Mucositis prevention, hematologic growth factors, parental nutrition and all other supportive care were identical during the two PBSCT and followed institutional protocol. Regimen-related toxicity, particularly mucosal toxicity were compared between the first and second PBSCT with each patient representing its own control. Oral mucositis was assessed according to the WHO oral-tissue toxicity scale. **Results.** Palifermin use during the second PBSCT prevented the occurrence of oral mucositis in all 5 patients, in comparison to the first PBSCT where WHO oral mucositis of grade 3, 2 or 1 in severity was recorded. **Conclusion.** The observation of complete mucositis prevention during the second PBSCT warrants further evaluation in larger but similar populations would be of interest to confirm these encouraging results.

**Table 1. Characteristics of patients and side effects after double transplantation procedure.**

<table>
<thead>
<tr>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnosis</td>
<td>Myeloma IgG III</td>
<td>Myeloma IgG III</td>
</tr>
<tr>
<td>Interval between PBSCT’s</td>
<td>123 days</td>
<td>120 days</td>
</tr>
<tr>
<td>PBSCT1</td>
<td>PBSCT2</td>
<td>PBSCT1</td>
</tr>
<tr>
<td>CD 34+ infused</td>
<td>3.2×10(^6)/kg</td>
<td>3.2×10(^6)/kg</td>
</tr>
<tr>
<td>Hospitalization post PBSCT</td>
<td>21</td>
<td>14</td>
</tr>
<tr>
<td>TPN</td>
<td>21</td>
<td>14</td>
</tr>
<tr>
<td>WHO Grade Oral Mucositis</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>WHO Grade pyrosis, dyspnea, esophagitis</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>WHO abdominal pain, colitis</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>WHO diarrhea</td>
<td>0</td>
<td>3</td>
</tr>
</tbody>
</table>

**1115**

**LINEAGE SPECIFIC CHIMERISM ANALYSIS ALLOWS EARLY DETECTION OF RELAPSES AFTER ALLOGENEIC STEM CELL TRANSPLANTATION**

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**Backgrounds.** Chimerism analysis is essential to verify the origin of hematopoiesis after allogeneic stem-cell transplantation (SCT). Considering that after SCT, almost all relapses are recipient-derived, the reappearance of mixed chimerism or an increasing fraction of recipient-derived cells should prompt the suspicion of relapse and be differentiated from graft failure or rejection. Furthermore, as reduced intensity conditioning SCT (RIC-SCT) emerge as a frequent procedure, a correct interpretation of chimerism analysis becomes imperative since transient mixed chimerism is frequently observed after RIC-SCT and does not necessarily mean an unwanted evolution. **Aims.** To evaluate the usefulness of our methodology of lineage-specific chimerism analysis to sensitively detect relapse early after conventional or RIC SCT. **Methods.** We performed chimerism analysis in whole peripheral blood (PB) as well as in the separated cells on days 14, at the time of neutrophil recovery and monthly thereafter during the first year after SCT. Chimerism was determined on PB by short tandem repeat (STR) analysis on unfractionated PB or after cell separation (lineage-specific chimerism: positive selection of mononuclear cells using CD3, CD19 and CD16 monoclonal antibodies conjugated with magnetic beads; Dynabeads®). DNA was obtained with the Miller method and samples were used in a multiplex polymerase chain reaction to amplify 6 (D8S1130, D21S1270, D6S1031, D3S1358, D1S160, D15S150, D18S51). Primers were marked with Cy5. Separation and detection of fragments were done with ALF-Express® and infomingative peaks were analyzed with the AlleleLink® software. Depending on the locus, sensitivity to detect mixed chimerism was evaluated in 1 to 5%. **Results.** Fifteen patients were allografted at Maciel Hospital, Monientevo, Uruguay, from January 2005 to December 2004 and those with at least
I chimerism analysis were included (n=13). Five patients relapsed during the first year after SCT. Three of them were induced by chimerism analysis: in one case, mixed chimerism was observed in the subpopulation compromised by the disease (CD19+ in B lineage ALL with CD19 positive blasts) while in the other 2 patients, relapses were detected by an increasing recipient hematopoiesis in unfractonated blood and CD3-, CD19-CD15 subpopulations (AML with CD15+, CD8- and CD19-blasts and ALL with CD19+, CD8- and CD15+ blasts). The other 2 patients had relapses of CML that were detected by nested PCR for bcr/abl and cytogenetic analysis but did not show mixed chimerism. Conclusions. These results suggest that, at least in some diseases, lineage specific chimerism could be an alternative to other methods to increase sensitivity and specificity of relapse detection.

1116 ATORVASTATIN INHIBITS THE EXPRESSION OF ADHESION MOLECULES ON ENDOTHELIAL CELLS BY REDUCING REACTIVE OXYGEN SPECIES

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Backgrounds. The migration of circulating monocytes into the subendothelial occurs through the expression of some adhesion molecules on endothelial cells. The nuclear factor (NF)-κB, a redox-sensitive element, plays a key role in adhesion molecule gene induction. Aims. Was wished to explore further the possibility that other types of peripheral blood cells might do so as well.

We have established that the majority of Treg cells in the peripheral blood of normal human donors express TGF-βRII complexes. Recently, several groups have identified TGF-βRI on the surface of the immunoregulatory CD4+CD25+ Treg (Treg) cells in most of a few of these adhesion molecules that have been identified as targets for oxidative stress. Aims. Was to investigate the expression of TGF-βRII and TGF-βRII on target cells, these receptors deliver a negative or positive signal that prevents or activates the TGF-β-mediated lysis of target cells. NK cells and CIK express various TGF-β receptors. There are some reports that expression of these receptors could be increased in neoplasms. Aim. The aim of this study was to investigate the expression of TGF-βRII (CD152b) and TGF-βRII (CD254) in NK cells and CIK in multiple myeloma (MM) and non-Hodgkin's lymphoma (NHL) patients. Methods. 41 MM (27 Female, 14 Male, mean age: 64 years) and 38 NHL (19 Female, 19 Male, mean age: 59 years) patients were studied. They were studied at diagnosis and after 3 courses of chemotherapy. The control group consisted of 15 age-matched normal donors. The presence of CD152b and CD254 on NK and NK22A was evaluated in NK cells and CIK isolated from patients and normal donors. Peripheral blood mononuclear cells (PBMCs) were obtained by density-gradient centrifugation (Ficoll-Hypaque) of heparinized venous blood.

Table 1.

<table>
<thead>
<tr>
<th>TGF-βRII</th>
<th>TGF-βRII</th>
</tr>
</thead>
<tbody>
<tr>
<td>NK cells</td>
<td>NK cells</td>
</tr>
<tr>
<td>CD152b</td>
<td>CD254</td>
</tr>
<tr>
<td>[μg]</td>
<td>[μg]</td>
</tr>
<tr>
<td>30</td>
<td>55</td>
</tr>
<tr>
<td>75</td>
<td>42</td>
</tr>
<tr>
<td>44</td>
<td>22</td>
</tr>
<tr>
<td>30</td>
<td>62</td>
</tr>
<tr>
<td>19</td>
<td></td>
</tr>
</tbody>
</table>

MM 42 |
NHL 37 |

Conclusions. These results show that TGF-βRII expression is a useful marker for Treg cells in the peripheral blood of normal human donors expressing TGF-βRII on their surface, and wished to explore further the possibility that other types of peripheral blood cells might do so as well.

Methods. Peripheral blood from healthy normal donors (n=16, 5M 11F, aged 20-45) having no first or second degree relatives suffering from autoimmune diseases, was obtained and pelleted by centrifugation (1200 rpm for 10 min). The supernatant was then counted microscopically. Three colour immunofluorescence staining was performed. Seven ml of monoclonal antibodies conjugated with FITC, PE and Cy-Chrome was added to each tube. We used the following monoclonal antibodies specific for: CD14, CD45 (DAKO, Denmark), CD8, CD56, CD94, CD152b and NK22A (Becton Dickinson, USA). Tubes were then agitated and incubated for 20 min. at 4°C in the dark, after which 5 mL of PBS-Ca2+Mg2+ containing 0,1% NaN3 paraformaldehyde. 20 000 labelling events were routinely accumulated and analysed for fluorescence on PAS flow cytometer (Partec, Munster, Germany) using FloMax software. The results were statistically analysed using test ANOVA rang Kruskal-Wallis. Results. Results are showed in the Table 1. Conclusion. We have demonstrated...
that there are no differences in distribution of CD15b and CD94/NKG2A in NK and CIK in MM and nHL compared with normal donors. We have showed that the mean percentage of NK and CIK with CD94 expressing NKG2A is lower in MM patients compared with normal donors (p<0.05). It means that there is an increased expression of non-functional CD94 on NK and CIK of myeloma patients.

1119
UNEXPECTED ANTAGONISTIC EFFECT OF RITUXIMAB WITH PROCARBAZINE DISCLOSED DURING AN IN VITRO TESTING OF RITUXIMAB-MEDIATED SENSITIZATION OF B-CELL LINES TO COMMONLY USED ANTICANCER DRUGS

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Backgrounds. Rituximab is a chimeric monoclonal antibody specific to the CD20-antigen expressed on mature B-lymphocytes. The antibody sensitizes lymphoma cells to differently acting cytotoxic drugs. Although some combinations of cytostatic agents with rituximab have already been tested, there are many others for which no information is available. Aims. To analyse some commonly used and some new combinations of rituximab with differently acting cytotoxic agents in vitro using permanently transformed B-cell lines. Methods. The stable cell lines derived from a follicular lymphoma (WSU-NHL, DHL-4 and DOHH-2) and Burkitt lymphoma (RAMOS) were used for an in vitro viability assay. The cell lines were pretreated by 20 µg/ml of rituximab for 72 hours, followed by a subsequent incubation with the cytotoxic drugs (fludarabine, doxorubicin, vincristine, dexamethasone and procarbazine in four different concentrations) for 48 hours. A proliferation activity was estimated using a WST-1 assay. Obtained data were statistically evaluated using multi-way analysis of variance with interactions. The concentrations, presence or absence of pretreatment and plate variability were taken for the fixed effect. A cell cycle after the rituximab pre-treatment was analysed by flow-cytometry with propidium iodide. Results. Rituximab significantly decreased an S-phase of the DHL-4 cells, while no prominent effect on cell cycle was observed for the other cell lines. We observed a significantly different sensitivity of follicular lymphoma and Burkitt’s lymphoma cells to vincristine and fludarabine (FL cells were highly sensitive to vincristine and rather poorly to fludarabine, while an opposite effect was seen for BL cells). The rituximab pretreatment sensitized all cell lines to vincristine, while none were sensitized to doxorubicin. Heterogenous results were obtained for the other combinations. A statistically significant influence of the rituximab pretreatment was proved for: dexamethasone at DOHH-2 and RAMOS, Fludarabine at WSU-NHL and fludarabine and dexamethasone at DHL-4 cell lines. We obtained quite unexpected results for procarbazine in combination with rituximab. Although the drug strongly inhibited a metabolic activity in all tested cell lines, the effect was just opposite when the cells were pre-treated with rituximab. A highly statistically significant antagonistic effect of rituximab was proved for all the cell lines. Summary. The data confirm that rituximab might sensitize lymphoma B-cells to most of differently acting anti-cancer agents. There are, however, some drugs manifesting a strong antagonistic effect with respect to rituximab. Therefore, based on our experimental data, the combination of rituximab with chemotherapeutic agents containing procarbazine (e.g. R-COP) does not seem to be clinically warranted.

1120
ASPIRIN RESISTANCE IN PATIENTS AFTER ISCHAEMIC STROKE AND ISOPROSTANE (8-EPProstaglandin F2α) PLASMA CONCENTRATION

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Background. The limited efficacy of secondary prevention for ischaemic stroke may be partially related to aspirin resistance leading to continuous generation of intraplatelet thromboxane A2. Besides other underlying metabolic mechanisms, an oxidant stress along with nonenzymatic biosynthesis of isoeicosanoids supporting the platelet activation has been suggested. Aims. We analysed the incidence of aspirin resistance in survivors of ischemic stroke and compared the usefulness of some platelet tests designed for its laboratory exploration. Material and Methods. Forty four patients, at least a month after acute onset of ischaemic stroke were included into the study. All of them have been receiving 75-150 mg aspirin daily at least for a month. The control group consisted of 12 adequately matched healthy volunteers. The platelet function was investigated by platelet aggregation induced by either ADP (3.5 and 5.0 µM), collagen (2 µg/ml) or arachidonic acid (AA) (0.6 mM) and measurement of closure time on the collagen and epinephrine (Col/Ep) cartridge in PFA-100® analyzer. Thromboxane A2 metabolite - 11-dehydro Thromboxane B2 (11-dTxB2) and Prostaglandin F2α (8-epi Prostaglandin F2α) plasma concentration by immunoenzymatic method (ELA Kits from Cayman Chemicals) were also determined. The aspirin ingestion was controlled by diminished intraplatelet concentration of malondialdehyde. Aspirin resistance has been determined by the following criteria: the intensity of platelet aggregation induced by ADP 60%, collagen 60%, AA 20%, PFA-100® closure time >165 s and as reference indicator; 11-dTxB2 concentration mean of the control group minus SD.

Table 1. Aspirin resistance in patients after stroke.

<table>
<thead>
<tr>
<th>ADP</th>
<th>PFA-100</th>
<th>11-dTxB2</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.5 µM</td>
<td>5.0 µM</td>
<td></td>
</tr>
<tr>
<td>45%</td>
<td>52%</td>
<td>20%</td>
</tr>
</tbody>
</table>

Statistically significant inverse correlations have been found between the plasma concentration of 11-dTxB2 and PFA-100® (Col/Ep) closure time (r=-0.31; p<0.039) as well as between plasma concentration of 8-epiPGF2α and PFA-100® closure time (r=-0.36; p<0.019). Conclusions. 1. Laboratory tests reveal aspirin resistance in almost half of patients after ischaemic stroke. 2. The most significant correlation has been found between plasma concentration of reference indicator 11-dehydro Thromboxane B2 and PFA-100® closure time. 3. An important interrelationship observed between PFA-100® closure time and plasma concentration of 8-epi Prostaglandin F2α may support the hypothesis of nonenzymatic production of isoprostanoids with platelet proaggregatory activity, playing a role in aspirin resistance.

1121
INDUCTION OF APOPTOSIS IN NB4 CELL LINE TREATED WITH ARSENIC TRIOXIDE AND THE EFFECT OF VIT.D3 ASSESSED BY THE COMET ASSAY

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Tarbiat Modares University, TEHRAN, Iran

Backgrounds. Successful treatment of acute promyelocytic leukemia (APL) relies on the ability to kill or arrest the growth of the leukemic blasts, which can be accomplished by inducing maturation, as in the case in differentiation therapy by the conventional chemotherapy by induction of Apoptosis. NB4 cells, a model of APL, have shown to undergo mono- cytic differentiation in response to 1α, 25 dihydroxy Vitamin D3 (1α, 25 D3) and apoptosis or partial differentiation in response to arsenic trioxide (AS2O3). A change that usually happens during apoptosis is the severe fragmentation of cellular DNA, a characteristic that can be readily measured by single cell gel electrophoresis, known as the comet assay. Aims. Study of the effects of arsenic trioxide (ATO) and Vit.D3 on induction of apoptosis in NB4 cell line using the neutral comet assay. Methods. NB4 cells were treated with various doses of arsenic trioxide (0.1-8 µmol) and Vit.D3 (100-600 nM) alone or combined together. 24 hours later cells were mixed with low melting point agarose and placed onto a pre-coated slide. After lysis and electrophoresis in neutral condition, cells were stained with ethidium bromide and observed under a fluorescent microscope. The data were then analyzed and compared. Results. Show that ATO induced apoptosis in NB4 cells at all doses used in this study. The effect was dose and time dependent and significantly different from controls (p<0.05). In contrast, Vit.D3 at concentrations of 100-600 nM showed no effect on induction of Apoptosis. Treatment of the NB4 cells with arsenic trioxide in combination with Vit.D3, a monocytic inducer, resulted in reduction of apoptosis as compared to arsenic trioxide alone at the same concentration (p<0.05) in all groups. Conclusion: Results show clearly that ATO is a potent inducer of apoptosis in NB4 cells and the effect is dose and time dependent. On the other hand, the results suggest that Vit.D3 decreases the sensitivity of cells to arsenic trioxide. A significant decrease in apoptosis in the various treatment groups, clearly gives evidence that Vit.D3 has a protective role (in this combination). Also neutral comet assay can be considered as a suitable
Translocations involving the RARA locus on 17q21 have been identified in acute promyelocytic leukemia (APL). The majority of APL harbors the t(15;17)(q22;q21), resulting in a PML-RARA fusion transcript. Variant rearrangements involving RARA in APL are t(11;17) (p36;q21)/RARA, t(5;17)(q35;q21)/RARA, t(4;17)(q23;pter)/RARA, and t(11;17)(p13;q11)/RARA. Among the 46,XY,t(10;11)(q23;p15) in 15/15 metaphases. To study NUP98 involvement in relapse of acute myeloid leukemia. Bone marrow karyotype was normal. The cytogenetic analysis suggested the presence of a pseudodiploid clone. The presence of pseudodiploidy or hypodiploidy correlated in general with moderate or poor outcome. Of total survival time (pST) was the highest in the groups of children with hypodiploidy >50 chromosomes or with TEL/AML1 fusion, both without other changes, structural or numerical. Moreover, TEL/AML1 was a good prognostic factor only when present in a high percentage (>80%) of examined cells. The presence of pseudodiploidy or hypodiploidy correlated in general with moderate or poor outcome. The outcome for a group of patients with one of the following: t(1;19), t(9;22) or 11q22 rearrangement, was the worst and pEFS significantly lower than in the remaining patients (p<0.05). The most unfavorable independent risk factors were MLL rearrangements and BCR/ABL. The presence of MLL rearrangements caused 12-times increased, and 3-times increased risk of relapse or treatment failure. The WBC and early response to induction therapy were significant (p=0.05), independent hematological and clinical risk factors in ALL patients. The results of the study confirm the prognostic value of cytogenetic, FISH and molecular analyses in childhood ALL and underline the need of using them together at the diagnosis of ALL to establish the prognosis of the disease.

ACKNOWLEDGMENTS
Acknowledgements. We wish to thank Dr M. Rocchi (University of Bari, Italy) for providing DNA RP41 and RPS3 clones.
reduction [DHR test] by PMA-stimulated neutrophils as an initial diagnostic test by Flowcymetry. Genomic DNA was isolated from peripheral blood leukocytes of the affected patient and two siblings and his father by Qiagen DNA extraction Kit. Analysis for the presence of specific genomic coding sequences in several genes at the Xp21 locus namely DMD exon 59; PRKG1 exon 1; XK exons 1,2,3; CYBB exon 10; TCTE1L exon 5; SRFX exon 1; RPGR exon 19 and OTC exon 1 were amplified by PCR reaction using specific primers. The presence of expected PCR products were reconfirmed by and documented by gel electrophoresis using an internal control gene. Additional studies were also performed to evaluate the Kell antigen system on the red blood cells. Results. DHR test showed no oxidative burst consistent with the diagnosis of CGD.

It was observed that the patient had a large deletion extending from 5PRKG1 to TCTE1L genes (Figure) with loss of both XK and CYBB genes. Flow cytometry showed weak expression of Kell antigens on the red blood cells of the patient. Summary/Conclusions. This study illustrates the rare event in our patient presenting with clinical manifestations of CGD and McLeod’s syndrome owing the underlying deletion of 5PRKG1 to TCTE1L genes.

1126
IDENTIFICATION OF THE V617F JAK2 MUTATION IN MYELOPROLIFERATIVE DISORDERS
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Backgrounds. Polycythemia Vera (PV), Essential Thrombocytethemia (ET), Myelofibrosis (MF) and Chronic Myelogenous Leukemia (CML) were grouped into a spectrum of related disorders by Dameshek in 1951, dubbed Chronic Myeloproliferative Diseases (MPDs). However, a disease-causing genetic alteration (BCR/ABL rearrangement) has been identified only in CML. The other 3 disorders (ET, PV and MF) are classified as the BCR/ABL negative classics MPDs and are therefore distinguished from CML. The discovery of a single mutation in the Janus Kinase (JAK)-2 gene (substitution of a valine for a phenylalanine in the codon 617) in a high percentage of cases of PV, ET, MF suggests that it may be the underlying molecular mechanism for these disorders. This single mutation has been reported in 65-97% of patients with PV, 25-57% of ET cases, 25-57% of MF cases and in 20% of patients with unclassified MPDs. The identification of JAK2 mutation represents a major advance in our understanding of the molecular pathogenesis of MPDs and provides a hallmark of genetic alteration in these disorders. Aims and Methods. We studied 57 Portuguese patients with MPD: 32 patients with MPD-NOS (not otherwise specified), 11 with PV, 11 with ET and 3 with idiopathic MF. In each case, DNA obtained from bone marrow or peripheral blood cells was amplified by PCR using specific primers for exon 12 of the JAK2 gene. The mutation V617F was detected by RFLP. Results. Analysis of 32 MPD-NOS revealed in 11 (46.9%) the V617F mutation. This mutation was also identified in 72.7% cases of PV and 27.3% of ET cases. V617F mutation was not identified in the 3 cases with idiopathic MF. Conclusions. The results of our study are in keeping with published reports, showing that V617F mutations are very frequent in MPDs, mainly in PV and in ET. These data suggest that the V617F mutations participate in the pathogenesis of chronic MPDs and will probably lead to a new classification of these diseases, contribute to a better stratification of patients according to prognosis, and hopefully allow the development of novel therapeutic approaches.

1127
MOLECULAR DIAGNOSIS OF β-THALASSAEMIA IN ROMANIA: THE FIRST APPLICATION TO PRENATAL DIAGNOSIS
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Thalassemia major is a classical example of a disease that can be prevented by prenatal diagnosis. In Romania there are currently 300 patients with thalassemia major under the management of specialized institutions. So far, the prenatal diagnosis of thalassemia was not available in Romania, for various reasons. For the prenatal diagnosis of β-thalassemia the first step is the characterization of the spectrum of mutations causing this disease in the Romanian population. In 2003 our institution, benefiting from the help of the Romanian Academy of Science initiated a Screening Programme for thalassemia having as a main purpose to perform a screening for β-thalassemia mutations previously described in the Romanian population. METHOD: Haematological data were collected with automated cell counters (Coulter). Quantification of haemoglobin was done by cation exchange HPLC and by agarose gel electrophoresis. Analysis of the mutation in the β-globin gene has been performed by using the PCR based Methods. Amplification Refractory Mutation System (ARMS), restriction enzyme analysis and Denaturing Gradient Gel Electrophoresis (DGGE). Results. Until now we have identified 11 β-thalassemia alleles: IVS I-110 (37,88%), CD 39 (13,64%), IVS II-745 (13,64%), IVS I-1 (3,03%), IVS I-87 (8,03%), CD 5 (8,03%), CD 6 (8,03%), CD51 (1,51%), +22 (1,51%), polyA (1,51%).

Using this experience we were able to perform the first prenatal diagnosis for a young couple at risk for thalassemia major: maternal genotype IVS I-110 / Normal and paternal genotype IVS II-745 / Normal. Fetal samplings were collected by amniocentesis in the second trimester. Maternal contamination of the fetal DNA was ruled out by STR genotyping. Fetal genotype was IVS I-110 / IVS II-745 compatible with the presence of β-thalassemia major. These results were confirmed by the DNA analysis performed in National Thalassaemia Center from Athens, Greece. CONCLUSION: The results of this study point to a successful future prenatal diagnosis of β-thalassemia in Romania, using a rapid and accurate molecular method. Together with the implementation of proper preventive health measures and the education of the parents regarding their carrier status, we are hoping that this method will be used as the common application approach to decrease the incidence of thalassemia major.

Funding. This work was supported by grant 48 and 49/2005 CEEX.

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DEVELOPMENT OF A QUANTITATIVE METHOD FOR ASSESSMENT OF BCR-ABL TRANSCRIPTS BY TAQMAN TECHNOLOGY FOR THE LIGHTCYCLER INSTRUMENT
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APPLICATION TO PRENATAL DIAGNOSIS
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The common application approach to decrease the incidence of thalassaemia major is a classical example of a disease that can be prevented by prenatal diagnosis. In Romania there are currently 300 patients with thalassaemia major. Funding. This work was supported by grant 48 and 49/2005 CEEX.
The standard treatment of newly diagnosed multiple myeloma (MM) patients is based on induction treatment followed by high-dose melphalan. CR/NCR percentages range from 20-50% with event free survival (EFS) ranging from 18 months to 20 months. The 5-year survival rates are 25% to 50%, however all patients eventually relapse and succumb to the disease. In patients with unfavourable prognostic factors such as a high serum β2-microglobulin and/or a deletion of chromosome 13, or in elderly patients the prognosis remains poor. Recently, several promising new agents were developed, which interfere with critical cell-survival pathways in myeloma. Amongst these agents, Bortezomib, a proteasome inhibitor, and Thalidomide, an anti-angiogenic and immunomodulatory drug, have a remarkable effect in patients with relapsed or refractory MM with 30-40% response rates. In combination with Dexamethasone and/or other conventional agents overall response rates of 50-70% can be achieved. In newly diagnosed patients the responses vary from 70 - 85%. Moreover, Bortezomib was found to overcome poor prognostic factors like a high β2-microglobulin and/or deletion of chromosome 13. However, 15-30% of newly diagnosed patients do not respond to Bortezomib or Thalidomide. Secondly, 30% of the patients treated with these novel agents have to stop prematurely because of intolerable side effects, such as polyneuropathy, thrombocytopenia, thrombosis and gastrointestinal symptoms. To gain new insights into the mechanisms of drug response and toxicity associated with these agents, we have embarked on a prospective study to analyze gene expression profiles of myeloma specific genes in plasma cells purified from bone marrow from myeloma patients at diagnosis who have been treated with these novel agents in order to learn which genes govern the response, PFS and OS upon treatment with Bortezomib and Thalidomide. Gene expression profiling of CD138 magnetic cell selected (MACS) myeloma plasma cells will be performed using Affymetrix GeneChip Human Genome U133 plus 2.0 arrays. In addition, we will perform a Single Nucleotide Polymorphism analysis of germline DNA samples. For the statistical analysis we will use eQTL analysis and multifactorial analysis with the clinical data set from these patients. We will present the initial results including an unsupervised cluster analysis based on the array results from the first cohort of 50-75 patients.

1129
TRANSCRIPTIONAL PROFILING OF EPSTEIN-BARR VIRUS (EBV) GENES AND HOST CELLULAR GENES IN NASAL NK/T-CELL LYMPHOMA AND CHRONIC ACTIVE EBV INFECTION
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Backgrounds. Nasal NK/T-cell lymphomas is an aggressive subtype of non-Hodgkin lymphoma (NHL) that is closely associated with Epstein-Barr virus (EBV). The clonal expansion of EBV-infected NK or T cells is also seen in patients with chronic active EBV (CAEBV) infection, suggesting that two diseases might share a partially similar mechanism by which EBV affects host cellular gene expression. Aim. To understand the pathogenesis of EBV-associated NK/T-cell lymphoproliferative disorders (LPD) and design new therapies. Methods. We employed a novel EBV DNA microarray (HHV-4 Virchip) to compare patterns of EBV expression in six cell lines established from EBV-associated NK/T-cell LPD. We also analyzed the gene expression patterns of host cellular genes using an Affymetrix U133plus2.0 chipset. Results. We found that expression of BZLF1, which encodes the immediate-early gene product Zta, was expressed in SNK/T cells. We identified a subset of pathogenically and clinically relevant host cellular genes which might be a putative contributor for tumor progression. CONCLUSION. This study describes a new approach to understanding the relationship between serine/threonine protein phosphatase inhibitors
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GENE EXPRESSION PROFILING AND DETERMINATION OF GENETIC HETEROGENEITIES AS PROGNOSTIC FACTORS IN PATIENTS WITH MULTIPLE MYELOMA TREATED WITH CONVENTIONAL VERSUS NOVEL AGENTS IN CORRELATION WITH OUTCOME
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The standard treatment of newly diagnosed multiple myeloma (MM) patients is based on induction treatment followed by high-dose melphalan. CR/NCR percentages range from 20-50% with event free survival (EFS) ranging from 18 months to 20 months. The 5-year survival rates are 25% to 50%, however all patients eventually relapse and succumb to the disease. In patients with unfavourable prognostic factors such as a high serum β2-microglobulin and/or a deletion of chromosome 13, or in elderly patients the prognosis remains poor. Recently, several promising new agents were developed, which interfere with critical cell-survival pathways in myeloma. Amongst these agents, Bortezomib, a proteasome inhibitor, and Thalidomide, an anti-angiogenic and immunomodulatory drug, have a remarkable effect in patients with relapsed or refractory MM with 30-40% response rates. In combination with Dexamethasone and/or other conventional agents overall response rates of 50-70% can be achieved. In newly diagnosed patients the responses vary from 70 - 85%. Moreover, Bortezomib was found to overcome poor prognostic factors like a high β2-microglobulin and/or deletion of chromosome 13. However, 15-30% of newly diagnosed patients do not respond to Bortezomib or Thalidomide. Secondly, 30% of the patients treated with these novel agents have to stop prematurely because of intolerable side effects, such as polyneuropathy, thrombocytopenia, thrombosis and gastrointestinal symptoms. To gain new insights into the mechanisms of drug response and toxicity associated with these agents, we have embarked on a prospective study to analyze gene expression profiles of myeloma specific genes in plasma cells purified from bone marrow from myeloma patients at diagnosis who have been treated with these novel agents in order to learn which genes govern the response, PFS and OS upon treatment with Bortezomib and Thalidomide. Gene expression profiling of CD138 magnetic cell selected (MACS) myeloma plasma cells will be performed using Affymetrix GeneChip Human Genome U133 plus 2.0 arrays. In addition, we will perform a Single Nucleotide Polymorphism analysis of germline DNA samples. For the statistical analysis we will use eQTL analysis and multifactorial analysis with the clinical data set from these patients. We will present the initial results including an unsupervised cluster analysis based on the array results from the first cohort of 50-75 patients.
breast cancer cell line. The aim of our study is to investigate the potential role of serine/threonine protein phosphatase system and specific inhibitors of this system in docetaxel/paclitaxel induced cytotoxicity on HL60 cells. Materials and method. HL60 myeloid leukemia cell line was used as the model cell line. IC50 dose of paclitaxel and docetaxel were found to be 20 nM and 5 nM respectively by using trypan blue dye exclusion and XTT photometric assay. Protein phosphatase inhibitors showed significant increase in the taxan-induced cytotoxicity of HL60 cells. Acridine orange/ethidium bromide and Hoechst 33342-PI methods were used for evaluation of taxan-induced apoptosis of HL60 cells. Western blotting with specific antibodies against protein phosphatases was used to determine the changes in the expression of protein phosphatases after incubation of cells with taxans. Protein phosphatase activities were assessed by using specific ELISA kits. Results. Treatment of HL 60 cells with docetaxel and paclitaxel resulted in dose and time dependent cytotoxicity with 24 hours intervals. Combination studies of these drugs with phosphatase inhibitors showed significant increase in the taxan-induced cytotoxicity of HL60 cells. Acridine orange/ethidium bromide and Hoechst 33342-PI methods confirmed the taxan-induced apoptosis of leukemic cells. Protein phosphatase 1 and 2A activity was found to be increased after treating cells with docetaxel and paclitaxel at maximum level of 72 hour. Western blotting results showed the increase in the expression of protein phosphatase 1A catalytic subunit at 72 hours of incubation. Conclusion: Serine/threonine protein phosphatase system has significant role in taxan-induced cytotoxicity against leukemic cells. Potential use specific protein phosphatase inhibitors in combination with taxans will open new windows in the treatment of myeloid leukemias.

1132
A NEW FORMULA FOR DIFFERENTIATION OF IRON DEFICIENCY ANEMIA (IDA) AND THALASSEMIA TRAIT (TT)
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Introduction: The most commonly encountered disorders with mild microcytic anemia are iron deficiency anemia and thalassemia trait. Sensitivity and specificity of many different indices have been reported using red blood cell indices. Youden's index provides an appropriate measure of validity of a particular technique or question by taking into account both sensitivity and specificity. We compare the Youden's index for these indices as well. Methods. We studied 284 individuals with microcytic anemia aged between 6 month and 75 years. There were 188 females and 96 males involved in our study with mean age equal to 24.23(SD, 15.44). Ferritin, HbA2, and Complete Blood Cell (CBC), in which RBC, hemoglobin (Hb), Hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin(MCH), and Mean Corpuscular hemoglobin concentration (MCHC), were measured for all the participants. We diagnosed individuals with HbA2>3.4% as patients with β thalassemia (BT) and those who have a serum ferritin <12ng/ml or respond to administered iron and anemic situation in their blood subsides as patient suffering from Iron deficiency anemia (IDA). England Index, Mentzer Index, Srivastava Index, Kawakami Index, have been calculated for all formulas as well as OUR INDEX (Ehsani Index) = MCV-(10*RBC) Blood Counts were obtained by H1 Technicon Cell Counter System while ferritin was measured and HbA2 value determined by electrophoresis. Sensitivity, specificity, Positive IDA Predictive Value (PPV), Negative IDA (BT) Predictive Value (NPV), and Youden Index (YI) was calculated. Results: Considering the above criteria we diagnosed 130 patients with BT and 154 patients with IDA. Sensitivity and specificity for England Index was 99.2 and 69.5, Mentzer Index 94.6 and 95.5, Srivastava Index 88.5 and 85.7, Kawakami Index 86.2 and 98.1, and Ehsani's Index 90.0 and 95.5. Conclusions: The most frequently encountered diseases with microcytic anemia are TT and IDA. Screening for these indices as well. Methods. We studied 284 individuals with microcytic anemia aged between 6 month and 75 years. There were 188 females and 96 males involved in our study with mean age equal to 24.23(SD, 15.44). Ferritin, HbA2, and Complete Blood Cell (CBC), in which RBC, hemoglobin (Hb), Hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin(MCH), and Mean Corpuscular hemoglobin concentration (MCHC), were measured for all the participants. We diagnosed individuals with HbA2>3.4% as patients with β thalassemia (BT) and those who have a serum ferritin <12ng/ml or respond to administered iron and anemic situation in their blood subsides as patient suffering from Iron deficiency anemia (IDA). England Index, Mentzer Index, Srivastava Index, Kawakami Index, have been calculated for all formulas as well as OUR INDEX (Ehsani Index) = MCV-(10*RBC) Blood Counts were obtained by H1 Technicon Cell Counter System while ferritin was measured and HbA2 value determined by electrophoresis. Sensitivity, specificity, Positive IDA Predictive Value (PPV), Negative IDA (BT) Predictive Value (NPV), and Youden Index (YI) was calculated. Results: Considering the above criteria we diagnosed 130 patients with BT and 154 patients with IDA. Sensitivity and specificity for England Index was 99.2 and 69.5, Mentzer Index 94.6 and 95.5, Srivastava Index 88.5 and 85.7, Kawakami Index 86.2 and 98.1, and Ehsani's Index 90.0 and 95.5. Conclusions: The most frequently encountered diseases with microcytic anemia are TT and IDA. Screening for these indices as well.

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α THALASSEMS IN BAHRAIN
K. Shome,1 S. Abuamer,1 A. Al Ajmi,1 N. Jassim,1 J. Saleh Ali,1 G. Ameen,2 K. Shari,1 N. Mahdi,1 S. Al Arrayed,1 A.A. Satir2
1Sab马力a Medical Complex, MANAMA, Bahrain; 2Pathology, CMMAS, Arabic Gulf University, MANAMA, Bahrain; 3Bahrain Defence Forces Hospital, WEST RIFFA, Bahrain

α-thalassemia is one of the commonest genetic disorders in the Arab Gulf region and its reported incidence varies from 15% to 50% in the different countries in this region. Despite this widespread occurrence there are few comprehensive studies that describe genotype-phenotype correlations in this disorder as observed in this geographic location. To correlate the genotypes of α-thalassemia in Bahraini subjects with the respective phenotypic characteristics. Forty α-thalassemia cases were selected from patients referred by participating hematologists for the investigation of anemia or unexplained microcytosis. The following tests were done for each patient: I) measurement of hemoglobin and red cell indices II) staining for HBH inclusions III) analysis of hemoglobin and quantitation of hemoglobin fractions by HPLC and IV) molecular genotyping using a PCR-based strategy to identify the four common α-thalassemia haplotypes prevalent in the region (αα-, αααα, ααHph and αTSaudi). The assessment of clinical severity was based on the degree of anemia, the requirement for and number of episodes of transfusion and age at first transfusion. The αTSaudi/haplotype was the most common with a frequency of 41.9% among all haplotypes. This was followed successively by αααα(37.8%), ααHph(10.8%) and ααααHph(9.5%). The homozygous αTSaudi genotype was characterized by presence of high numbers of cells containing intraerythrocytic inclusions of HBH with typical morphology, markedly altered erythrocyte indices especially the RDW, high levels of hemoglobin (Hb) Bart’s and/or HbH4 and greater clinical severity. The other genotypes showed overlapping phenotypic features but none were severely affected. The homozygous αTSaudi abnormality is the only genotype in Bahrain with a distinctive phenotype that is identifiable by routine laboratory tests and accounts for almost all the severely affected cases. Premanual screening programs in the region should take these considerations into account when screening strategies are formulated.

1134
EFFICACY OF HYDROXYUREA (HU) IN REDUCTION OF PACK RED CELL TRANSFUSION REQUIREMENT AMONG CHILDREN HAVING β-THALASSEMA MAJOR: KARACHI HU TRIAL (KHT)
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Backgrounds. PRC transfusion and iron chelation remains the mainstay of treatment of β-thalassaemia major patients. HbF augmentation is the exciting new approach to treat haemoglobinopathy. Aims. This study evaluates the efficacy and safety of hydroxyurea in reducing volume of PRC transfusion in β-thalassaemia. Methods. 23 patients with β-thalassaemia major received HU mean dose, 16mg/kg/day. The results were evaluable after 24 months. Mean volume of PRC transfused was reduced in all. Mean PRC requirements for six months before starting HU was 2126.45 ml where as after 24 months on HU was 1489.59 ml (mean difference: 637.3 mL; 95% CI: 402.8 - 817.8; p<0.001). Interval between transfusions was increased by 68.7%. Mean increase was 12.1 days (CI: 10.8 - 13.4; p<0.001). Grade I myelosuppression was seen in four and diarrhea in two patients. Conclusion. Hydroxyurea was found to be a safe medicine in β-thalassaemia. It showed a reduction in transfusion requirement and increased interval between PRC transfusions.

1135
ENDOGENOUS ERYTHROPOIETIN PRODUCTION AND ERYTHROPOIETIC ACTIVITY IN ANEMIC CANCER PATIENTS WITH HEMATOLOGICAL MALIGNANCIES
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1Clinic for hematology, SKOPJE, Macedonia; 2Clinic for Hematology, SKOPJE, Macedonia

Backgrounds. Cancer anemia is multifactorial: blunted erythropoietin...
Hemoglobin raised or not omissions of such preparation lead to complications.

role of pre-operative transfusion practice in patients with SCA, whether undergoing general anesthesia and surgery. Patients with sickle cell anemia to receive red blood cell (RBC) transfusions before clinical manifestations. It is generally recommended for patients with sickle cell anemia to receive red blood cell (RBC) transfusions before undergoing general anesthesia and surgery. Patients with sickle cell anemia have increased chance of undergoing surgical procedures with higher morbidity. The practice of preoperative blood transfusion for such patients is still controversial. Lately, a great deal of controversial data accumulated in regards to transfusion management of such patients who require surgery. Aim. The aim of this prospective study was to assess the role of preoperative transfusion practice in patients with SCA, whether or not omissions of such preparation lead to complications.

### 1136

**THE SAFETY OF AVOIDING PRE-OPERATIVE TRANSFUSION IN PATIENTS WITH SICKLE CELL ANEMIA**

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King Abdulaziz University, JEDDAH, Saudi Arabia

**Backgrounds.** Sickle cell anemia (SCA) is a common hereditary blood disease seen in Saudi Arabia, the affected patients associated with severe clinical manifestations. It is generally recommended for patients with sickle cell anemia to receive red blood cell (RBC) transfusions before undergoing general anesthesia and surgery. Patients with sickle cell anemia have increased chance of undergoing surgical procedures with higher morbidity. The practice of preoperative blood transfusion for such patients is still controversial. Lately, a great deal of controversial data accumulated in regards to transfusion management of such patients who require surgery.

**Aim.** The aim of this prospective study was to assess the role of preoperative transfusion practice in patients with SCA, whether or not omissions of such preparation lead to complications.

**Methods.** A randomized study of 369 patients, median age 16-years-old (range: 1-58 years old) during the period between June 1996 and June 2001, underwent different surgical procedures at King Abdulaziz University Hospital and King Fahd Armed Forces Hospital. Surgical procedures included adenectomy, tonsillectomy, total lip arthroplasty, cholecystectomy, splenectomy, and Obstetric and Gynecological surgery. Patients were randomized into two groups: Group I (n=181), received no preoperative transfusion but were transfused compensated for blood loss during surgery. Group II (n=188) received simple or partial exchange transfusion preoperatively. All patients were clinically and hematologically stable in the immediate pre-operative period; also, were carefully hydrated and good oxygenation was maintained. Results. Results showed none of the patients developed major intra- or post-operative complications in both groups. 14.4% of the preoperative transfusion group developed post-operative complications in comparison to 7.2% in non-transfused group with a significant p value (0.002). Conclusion. Avoidance of preoperative transfusion is a safe practice in properly selected steady state sicklers. On the contrary, it is believed that the risks associated with transfusion were avoided.

### 1137

**PHARMACOKINETICS OF ERYTHROPOIETIN PRODUCED BY A HUMAN CELL LINE (EPOETIN Δ): SUBCUTANEOUS VS. INTRAVENOUS DOING IN PATIENTS WITH CHRONIC KIDNEY DISEASE**

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Shire Pharmaceuticals, WAYNE, USA

**Backgrounds.** Epoetin Δ (Dynepo™ - Shire) is an erythropoietin produced by gene-activation technology in a human cell line. As a result, it contains very few of the highly immunogenic Neu5Gc residues that are more commonly found in other recombinant erythropoietins. In renal anemia, the preferred route of administration for erythropoietin (either subcutaneous [sc] or intravenous [iv]) often differs depending on the status of the patient, the erythropoietin used and local practice. **Aim.** To assess the pharmacokinetics of iv or sc epoetin delta in patients with anemia and end-stage renal disease requiring dialysis. Methods. Patients with end-stage renal disease requiring dialysis who had been receiving epoetin α for at least 90 days entered a 1-week washout phase during which they did not receive recombinant erythropoietin. Patients were then randomized to one of four groups receiving single doses of epoetin delta 150 IU/kg (iv or sc) or 300 IU/kg (iv or sc). Blood samples were drawn before and for 72 h after administration. Results. In total, 28 patients entered the washout phase and 22 of these (12 men, 10 women) went on to receive epoetin Δ and complete the study. Pharmacokinetic parameters are shown in Table 1.

<table>
<thead>
<tr>
<th>Complications</th>
<th>Group I (N=181)</th>
<th>Group II (N=188)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Painful Crises</td>
<td>3</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Neurological Complication</td>
<td>0</td>
<td>4*</td>
<td></td>
</tr>
<tr>
<td>Minor Respiratory Complication</td>
<td>5</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Respiratory Distress (Atelectasis)</td>
<td>0</td>
<td>3*</td>
<td></td>
</tr>
<tr>
<td>Circulatory Overload or Heart Failure</td>
<td>0</td>
<td>5*</td>
<td></td>
</tr>
<tr>
<td>Infection</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Total (Percentage)</td>
<td>13 (7.0%)</td>
<td>27 (14.0%)</td>
<td>0.002</td>
</tr>
<tr>
<td>Dealy of Surgery</td>
<td>1</td>
<td>45**</td>
<td>&gt; 0.001</td>
</tr>
</tbody>
</table>

**Konclusion.** Bioavailability of sc epoetin Δ was 26-36% of that of iv epoetin Δ. The half-life of sc epoetin delta was approximately 30 h compared with 10-18 h for iv epoetin Δ. Treatment-emergent adverse events occurred in 45% of patients, but none of these were considered by investigators to have any relation to epoetin Δ. Conclusions. As expected, the pharmacokinetics of epoetin Δ differ depending on route of administration. The half-life of epoetin Δ in patients with renal anemia may be slightly higher than that reported for epoetin α (a half-life as low as 4 h has been reported), suggesting that longer dosing intervals may be possible with this agent.
1138
TWO NOVEL G6PD VARIANTS, G6PD PEDIOSIS-CIKARO AND G6PD PIOTRKOW IN POLAND
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1Institute of Biochemistry and Biophysics, WARSAW, Poland; 2Inst. of Haematology & Blood Transfusion, WARSAW, Poland; 3Pomeranian Medical University, Szczecin, Poland

Backgrounds. Glucose-6-phosphate dehydrogenase (G6PD) is the key enzyme of the pentose phosphate pathway whose main physiological function in red blood cells is to produce the NADPH, essential for the protection of the cells against oxidative stress. The majority of people with G6PD deficiency are asymptomatic but they may develop acute hemolytic anemia in association with infections or following the ingestion of certain drugs or fava beans. In some sporadic cases G6PD deficiency is the cause of chronic non-spherocytic haemolytic anemia, CNSHA. Aims. The aim of our study was to elucidate the molecular basis of G6PD deficiency. Methods. Genomic DNA was extracted from peripheral blood using standard methods. G6PD gene exons 2 to 13 were amplified by polymerase chain reaction (PCR). DNA fragments generated by PCR amplification were directly sequenced. The appropriate restriction enzyme analysis was used to verify the presence of found mutations. Molecular modeling of the ter-
reaction (PCR). DNA fragments generated by PCR amplification were

<16 years) comprised 38% of the study population. Mean baseline LIC was high in the overall population (Table 1), with approximately 80% of patients having a baseline LIC ≥ 7 mg Fe/g dw and the majority (68.6%) ≥ 10 mg Fe/g dw. Published data have linked LIC levels above ≥7 mg Fe/g dw with an increased risk of developing iron overload-related complications, primarily heart-related. Baseline serum ferritin levels were high and above clinically acceptable values. For most patients, transfusional iron intake was 0.3±0.5 mg/kg/day, corresponding to a mean daily amount of blood given of 0.3± 0.11 mL RBC/kg. In addition to this global analysis, local analyses by country have been performed. Conclusions. Baseline iron burden, as reflected by LIC and serum ferritin levels, was very high and above published clinically acceptable thresholds. This analysis demonstrates that despite the availability of chelation therapy, many patients were severely iron overloaded and therefore at high risk for developing complications. There were no differences between adult and paediatric patients. This suggests that patients were not receiving adequate chelation therapy to achieve iron balance. The development of a highly efficacious, well-tolerated and convenient iron chelator will improve compliance and allow physicians to use an effective chelation programme for their patients. This was a primary goal of the rigorous development programme for the once-daily, oral chelator deferasirox, which culminated in its registration with a broad indication by a number of health authorities.

Table 1. Patient demographics and baseline characteristics.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Median ± SD or n (%), n=615</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>19.0 ± 10.4</td>
</tr>
<tr>
<td>Patients aged 2 to &lt;16 years, n(%)</td>
<td>213 (37.6)</td>
</tr>
<tr>
<td>Patients aged ≥16 years, n(%)</td>
<td>384 (62.4)</td>
</tr>
<tr>
<td>Male: Female</td>
<td>299:316</td>
</tr>
<tr>
<td>Mean baseline LIC≥SD, mg Fe/g dw</td>
<td>127 (20.7)</td>
</tr>
<tr>
<td>All patients</td>
<td>127 (20.7)</td>
</tr>
<tr>
<td>2 to &lt;16 years</td>
<td>164 (10.0)</td>
</tr>
<tr>
<td>≥16 years</td>
<td>164 (10.6)</td>
</tr>
<tr>
<td>Baseline LIC category, n(%)</td>
<td>422 (68.6)</td>
</tr>
<tr>
<td>&lt;7 mg Fe/g dw</td>
<td>166 (27.0)</td>
</tr>
<tr>
<td>7 to &lt;10 mg Fe/g dw</td>
<td>66 (10.7)</td>
</tr>
<tr>
<td>≥10 mg Fe/g dw</td>
<td>2325 (58.1)</td>
</tr>
<tr>
<td>Mean baseline serum ferritin ≤ SD, ng/mL</td>
<td>106 (16.3)</td>
</tr>
<tr>
<td>All patients</td>
<td>2898±2099</td>
</tr>
<tr>
<td>2 to &lt;16 years</td>
<td>2870±1662</td>
</tr>
<tr>
<td>≥16 years</td>
<td>2914±2325</td>
</tr>
<tr>
<td>Mean transfusional iron intake ≤ SD, mg/kg/day</td>
<td>0.38±0.12</td>
</tr>
<tr>
<td>Iron intake category n (%)</td>
<td>0.38±0.12</td>
</tr>
<tr>
<td>0</td>
<td>5 (0.8)</td>
</tr>
<tr>
<td>0 to &lt;0.3 mg/kg/day</td>
<td>153 (24.9)</td>
</tr>
<tr>
<td>0.3-0.5 mg/kg/day</td>
<td>357 (58.1)</td>
</tr>
<tr>
<td>&gt;0.5 mg/kg/day</td>
<td>100 (16.3)</td>
</tr>
<tr>
<td>Mean blood given ≥ SD, mL RBC/kg/day</td>
<td>0.35±0.11</td>
</tr>
</tbody>
</table>

1139
BASELINE IRON STUDIES DEMONSTRATE SEVERE IRON OVERLOAD IN PATIENTS ENROLLED INTO THE DEFERASIROX (EXJADE, ICL670) CLINICAL TRIAL PROGRAMME
C. Kattamis, B. Meddebs, C. Ressayre-Djaffer, T. A. Balakina
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Backgrounds. Iron overload is a potentially life-threatening consequence of regularly inadequately chelated, mainly because of the demanding administration regimen of the reference standard chelator deferoxamine (DFO, Desferal®), and therefore do not gain the full benefits of treat-
ment. Deferasirox (Exjade®, ICL670) is a novel, once-daily, easily appli-
cable, oral iron chelator that is currently approved for use in eight coun-
tries, including the USA and Switzerland, in patients aged ≥2 years with chronic transfusional iron overload. To date, more than 1,000 patients have been enrolled into the deferasirox clinical trial programme. Aims. The primary aim of this cross-study analysis was to evaluate the severity of iron burden in patients prior to enrolment into the deferasirox clinical trial programme. Methods. Liver biopsy was performed at baseline and after 1 year in patients participating in two deferasirox clinical studies, 107 (n=495) and 108 (n=120). In Study 107, 248 patients with β-thalassemia (5, 10, 20 or 30 mg/kg/day) and 247 to DFO (<25, 25<≤<50, ≤50 and ≥50 mg/kg) according to baseline liver iron concentration (LIC). In Study 108, 67 patients with β-tha-
lassemia and 53 with other anaemias (eg myelodysplastic syndromes, Diamond-Blackfan anaemia, rare anaemias) were enrolled and received defer-
asirox. Liver biopsies were performed in 12 countries (Argentina, Brazil, Canada, Germany, France, Greece, Italy, Tunisia, Turkey, UK, USA). Results. In general, baseline characteristics were comparable between deferasirox and DFO cohorts. Paediatric patients (aged 1140
DETECTION OF RARE RHCE ALLELES IN COMORIAN INDIVIDUALS LIVING IN MARSEILLES, FRANCE
M. Touinssi, P. Baillly, J. Chiaroni
EFS-Alpes Maritime, MARSEILLES, France

Background. More than 70 000 comorians are living in Marseilles at present (10% of the total Comorian population has immigrated to France since 1970). Due to their genetic background and lack of data on this popula-
tion, some difficulties of transfusion are encountered. As described previously (Noizat et al. Blood, 2002), some rare RHCE phenotypes are found exclusively in black populations: i) RH : 18 (71A→G) with the three alleles (ceEK, ceAR, ceBI), ii) RH : 34 phenotype is produced by the (C)ces haplotype, iii) Partial Rhe : produced by the new (10% of the total Comorian population has immigrated to France currently inadequately chelated, mainly because of the demanding administration regimen of the reference standard chelator deferoxamine (DFO, Desferal®), and therefore do not gain the full benefits of treat-
ment. Deferasirox (Exjade®, ICL670) is a novel, once-daily, easily appli-
cable, oral iron chelator that is currently approved for use in eight coun-
tries, including the USA and Switzerland, in patients aged ≥2 years with chronic transfusional iron overload. To date, more than 1,000 patients have been enrolled into the deferasirox clinical trial programme. Aims. The primary aim of this cross-study analysis was to evaluate the severity of iron burden in patients prior to enrolment into the deferasirox clinical trial programme. Methods. Liver biopsy was performed at baseline and after 1 year in patients participating in two deferasirox clinical studies, 107 (n=495) and 108 (n=120). In Study 107, 248 patients with β-thalassemia were randomized to deferasirox (5, 10, 20 or 30 mg/kg/day) and 247 to DFO (<25, 25<≤<50, ≤50 and ≥50 mg/kg) according to baseline liver iron concentration (LIC). In Study 108, 67 patients with β-tha-
lassemia and 53 with other anaemias (eg myelodysplastic syndromes, Diamond-Blackfan anaemia, rare anaemias) were enrolled and received defer-
asirox. Liver biopsies were performed in 12 countries (Argentina, Brazil, Canada, Germany, France, Greece, Italy, Tunisia, Turkey, UK, USA). Results. In general, baseline characteristics were comparable between deferasirox and DFO cohorts. Paediatric patients (aged
ic primer PCR's were used to detect specific mutations corresponding to RHCE rare alleles and to determine their homozygous or heterozygous status. Results: Six individuals were found positive for the 712A→G mutation. Sequencing of RHCE exons 4, 5 and 6 showed that five individuals were heterozygous for c.eA and one was heterozygous for c.eK. 735G→C mutation, associated with antigens RH10 and RH20, was observed with high frequency (154 were positive and 58 were homozygous for the mutant allele). Twenty individuals carried the DCE(3)-8-D gene heterozygously, future sequencing will show if this hybrid gene, found in our Comorian population of Marseilles, is similar to the one found in African populations. Detection of 540IC→T is still under study. Nine individuals carried c.eMO heterozygously as they carried both T676 mutation and the wild-type G676. Furthermore, a new 902A→G (Ap301Gly) was detected and those cases were supposed to be specific to this Comorian population. The effect due to this particular mutation, which could be considered as a new RHCE variant, is not known yet. Conclu-
sions: These preliminary findings allowed us to calculate the incidence of the rare RHCE alleles and haplotypes in our population of 260 Como-
rian individuals as follows: c.eK: 0.3%; c.eA: 1.92%; no c.eB was found, c.eMO: 3.46%; (C)ces seemed to be present with a high frequency. This study showed that a variety of RHCE alleles are present in Comorian population of Marseilles. These data should contribute to define a particular strategy for transfusion in black populations.

Acknowledgments. We are grateful to F. Noizet-Pireme, MD, (EFS-Ile de France Paris, France) for her technical advice, and to Mr K. PAPA for referring to our laboratory the donor samples.

1142 MEASUREMENT OF ERYTHROCYTE BAND3 EXPRESSION IN HEREDITARY SCURFY SORCOSIS
S. Jacobsson, H. Johansson, G.L. Persson
 Sahlgrenska University Hospital, GOTTHUBEN, Sweden
Backgrounds. Hereditary spherocytosis (HS) is the most common inherited anemia among the spherocytic anemias, whereas autoim-
immune hemolytic anemia is the most common acquired. The prevalence of HS is not known, partly because of the heterogenous clinical mani-
festations. The most specific diagnostic techniques, i.e. erythrocyte pro-
tein analysis and molecular genetics are only provided by a few refer-
ner laboratories. In the routine setting diagnosis is based on typical family history, splenomegaly and jaundice and the finding of spher-
ocytes and reticulosis in the blood and increased osmotic fragility of the erythrocytes. The red cell anion exchanger band 3 is present in gross numbers in the erythrocyte cell membrane. In HS the expression of band 3 is diminished irrespective of the primary protein defect. The band 3 expression can readily be measured by flow cytomtery after labelling with eosin-5-maleimide (EMA) (King et al. 1999). Aim. Determine the diagnostic characteristics of the flow cytometric band 3 expression test. Methods. We have measured band 3 expression in 80 patients with HS, 10 patients with other hemolytic anemias and in 200 healthy volun-
teeers. We have also studied band 3 expression in patients with autoim-
immune hemolytic anemia, G-6-PD-deficiency, PK-deficiency, sickle cell ane-
mia and other rare forms of anemia. We have also studied the influence of recent transfusions and ongoing profuse hemolysis. Results. In our laboratory the cut off value for band 3 expression for diagnosing HS is 92.5% of that in non-HS persons. We confirm the findings of others that the band 3 expression is normal in all other forms of anaemia than HS. Ongoing hemolysis and recent transfusions can diminish the sensi-
tivity of the method. Conclusion. The flow cytometric measurement of band 3 expression has a high sensitivity and specificity for diagnosing hereditary spherocytosis and should be one of the primary investigations in cases of suspected hereditary spherocytosis.

1143 GLUCOSE TOLERANCE IN PATIENTS WITH β-TALASSAEMIA MAJOR
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Backgrounds. In chronically transfused thalassemic patients, whether insulin resistance is the primary abnormality leading to glucose metabo-
limism disturbances, or early introduced pancreatic damage and reduced insulin secretory capacity is the main cause of glucose intolerance, remains uncertain. Aims. To examine the incidence of glucose disturbances and endocrine co-morbidity in transfused patients with β-thalassaemia major. Methods. We assessed glucose responses during an oral glucose tolerance test in 243 regularly transfused thalassemic patients (107 males, 136 females, 25.26 ±6.2 years, age ± standard deviation, range 13-48). Patients’ records were thoroughly reviewed to determine the overall transfusional iron overload and start of chelation therapy (age and time of first blood transfusion/Chelation therapy. However, the development of glucose intolerance was significantly increasing with age (p<0.001). Except hypothyroidism, all the other endocrine complications were significant-
ly more frequent in patients with diabetes and IGT (Table).

<table>
<thead>
<tr>
<th>Cardiohypothyroidism</th>
<th>Hypoparathyroidism</th>
<th>Hypothyroidism</th>
<th>Osteoporosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.2% (24/197)</td>
<td>(21/197)</td>
<td>(129/197)</td>
<td>(56/197)</td>
</tr>
<tr>
<td>81%</td>
<td>(12/197)</td>
<td>(74/197)</td>
<td></td>
</tr>
<tr>
<td>3% (9/25)</td>
<td>28%</td>
<td>92%</td>
<td>36%</td>
</tr>
<tr>
<td>10.3%</td>
<td>(9/25)</td>
<td>(23/25)</td>
<td>(9/25)</td>
</tr>
<tr>
<td>Diabetes N=21</td>
<td>38.09%</td>
<td>42.85%</td>
<td>33.33%</td>
</tr>
<tr>
<td>8.7%</td>
<td>(8/21)</td>
<td>(7/21)</td>
<td>(1/21)</td>
</tr>
<tr>
<td>Fisher's Exact Test</td>
<td>p&lt;0.001</td>
<td>p=0.0015</td>
<td>p=0.0007</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p=0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p=0.0003</td>
</tr>
</tbody>
</table>
Conclusions. The degree of iron overload, at least as this can be reflected by ferritin levels, is not associated with the development of glucose intolerance. Long-term iron balance rather than the present iron status seems to be related to the development of glucose metabolic disorders. Physicians caring for patients with thalassemia major should be particularly alert to glucose intolerance since co-existence of other endocrine complications is common in these patients.

1144

EVILOATION OF THROMBOCYTOSIS RELATED TO IRON DEFICIENCY ANEMIA

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1Hopital Farhat Hached, SOUSSE, Tunisia; 2 Hopital Farhat Hached, SOUSSE, Tunisia

Backgrounds. The incidence and the outcome of thrombocytosis related to iron deficiency anemia is not described very well. The aim of this study was to perform an analysis of the evolution of thrombocytosis related to iron deficiency. We performed a retrospective analysis of 1570 consecutive patients with iron deficiency anemia collected between 1995 and 2005. Four hundred 40 patients (29%) had a thrombocytosis more than 500 × 10⁹/L and 2005. Four hundred 40 patients (29%) had a thrombocytosis more than 500 × 10⁹/L between Jan. 2005 and Dec. 2005. Six recipient/donor pairs are 6/6 HLA(A,B,DRB1) high resolution typing matched and five recipient/donor pairs are 1/6 HLA high resolution typing mismatched. Six children received peripheral blood stem cells transplantation (PBSC) and 6 children received bone marrow transplantation (BMT). β-thalassemia major children conditioned with BU 14-21 mg/kg+CY(140-200 mg/kg) + ATG(25-55mg/kg)+Flu(200 mg/m²). The graft contained median mononuclear cells 5.5 × 10⁹/kg; range 2.2 × 10⁹/kg -10.0 × 10⁹/kg and medi- can CD34+ cells 6.6 × 10⁹/kg range, 1.4 × 10⁹/kg - 22.5 × 10⁹/kg. All patients received CSA FK506, MMF (MTX) as graft versus host disease (GVHD) prophylaxis and hepargin + pGE1 as veno-occlusive disease (VOD) prophylaxis. Results. All of 11 children had got complete chimerism by the identification of FISH for XY chromosome or quantitative PCR for short tandem repeat (STR) and 10 (91%) children had thalassemia alpha after a median follow-up time of 5.8 months after transplant (range, 2-13 months). One child(9%) died from IV degrees acute GVHD. The ANC engrafted from +9 to +16 days and the time of platelets>20 × 10⁹/L was from +11 to +30 days. Five of eleven(45%) children suffered I-II degrees acute GVHD and 2/11 (18%) children (all under went PBSC) suffered I-II degrees acute GVHD. Complications included 2 case of infection in central nervous system(1 case of virus and 1 case of bacteria) and 4/11 (36%) cases of mild VOD. Conclusions. Our study showed higher incidence of severe aGVHD in unrelated PBSC for children with thalassemia major. Unrelated SCT can be alternative treatment for children’s β-thalassemia major in China

1147

PRETREATMENT WITH DASATINIB HAS NO ADVERSE INFLUENCE ON TRANSPLANT OUTCOME AFTER ALLOGENIC STEM CELL TRANSPLANTATION FOR PH+ CHRONIC MYELOGENOUS LEUKEMIA AND ACUTE LYMPHOBlastic LEUKEMIA

M. Leiba, A. Shimon, I. Hardan, R. Yenushalmi, A. Nagler
Chaim Sheba Medical Center, TEL HOMEMER, Israel

Allogeneic stem cell transplantation (alloSCT) is frequently used as salvage or curative therapy in patients with advanced chronic myelogenous leukemia (CML) or Ph+ acute lymphoblastic leukemia (ALL) who were previously treated with Imatinib. While one study showed higher incidence of GVHD, VOD and TRM, most studies have demonstrated that Imatinib therapy prior to alloSCT does not adversely affect transplantation outcome. We report three patients with advanced CML (n=2) and Ph+ ALL (n=1) who received Dasatinib prior to alloSCT from an HLA matched sibling (n=1) or mismatched related donor (HaploCT) (n=1). All patients received combination therapy with Dasatinib and Mycophenolate Mofetil (MMF) as post transplantation immunosuppression. All patients currently being used in patients with Imatinib-resistant advanced CML or relapsed/refractory Ph+ ALL. Most of these patients will eventually undergo alloSCT, raising the question of whether Dasatinib therapy may adversely affect transplantation outcome. We report three patients with advanced CML (n=2) and Ph+ ALL (n=1) who received Dasatinib prior to alloSCT from an HLA matched sibling (n=1) or mismatched related donor (HaploCT) (n=1). All were male with a median age of 28 (16 to 49) years. All three achieved complete hematological response, as well as complete (n=2) or partial (n=1) cytogenetic response prior to transplantation. They were conditioned with either a myeloablative protocol (n=1) or a reduced intensity protocol (n=1). GVHD prophylaxis consisted of CSA and MTX (n=2) or complete T cell depletion (n=1). Each patient received a mobilized peripheral blood stem cell graft with between 11.4 to 19.8 CD34+ cells/kg. All patients successfully engrafted reaching ANC > 0.5 × 10⁹/L on median day +11 and plt > 20 × 10⁹/L on median day +11. No patient developed unusual or severe toxicities including no hyperbilirubinemia or VOD. There was no increased risk of infection in the sibling transplants. No patient developed clinically significant GVHD. In this small number of patients with advanced CML and Ph+ ALL (the first to be reported receiving an alloSCT following Dasatinib therapy), we found no evidence that Dasatinib adversely affect transplantation outcome. Larger studies are obviously indicated to confirm our preliminary results.

1148

ADDITION OF CYTARABINE DOES NOT IMPROVE UPON MOLECULAR RESPONSES ACHIEVED BY IMATINIB ALONE IN CHRONIC MYELOGENOUS LEUKEMIA

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AIIMS, NEW DELHI, India

Backgrounds. The introduction of Imatinib, a targeted therapy for chronic myeloid leukemia (CML) has been one of the success stories of modern cancer management. However molecular techniques have shown that only 4-10% of Imatinib treated patients achieve complete molecular remission. Aim. To compare Imatinib alone with Imatinib -cytarabine to see if the combination could achieve greater molecular
responses. Methods. 85 newly diagnosed adult CML patients were ran- domized to receive Imatinib or Imatinib-cytarabine combination. Only those patients in chronic phase were included in the study. Hydroxyurea was the only therapy received by these patients prior to starting Imatinib. Imatinib was initiated at a dose of 400 milligrams, within 6 months of diagnosis. Cytarabine was added in 45 patients at a dose of 10 milligram /square meter for 10 days every month after 3 months of Imatinib. BCR-ABL transcripts were measured by real time PCR at baseline and at follow-up every 6 months. The reduction in BCR-ABL transcripts in the two groups was compared using Mann-Whitney test. Results. Patients were followed for a median period of 1.5 years (range 0.6-2.2 years) in the Imatinib group and 1.8 years (range 1.2-2.9 years) in the combination group. The baseline variables like age, Sokal risk groups and haemogram were similar in the two groups. Median values for age, haemoglobin, total leukocyte count and platelet count in the Imatinib group were 31 years (range 21-45 years), 11g/m% (range 5.6-15.4 g/m%), 150000/cubic millimeter (range 112000-242000/cubic millimeter) and 540000/cubic millimeter (range 125000-1126000/cubic millimeter) respectively. Male: Female ratio was 5:1 in Imatinib group and 5:1 in the combination group. All patients achieved complete haematological responses. Both groups tolerated their therapies equally well with no significant difference in toxicity profiles. 2 patients in each group discontinued therapy because of grade 4 cytopenias. 1 patient in the Imatinib group and 2 patients in the combination group discontinued therapy because of grade 4 skin toxicity. In the Imatinib group the median number of BCR-ABL transcripts at diagnosis was 345800 (range 605-1012561), which had reduced to 18286 (range 0-5412661) at follow-up, a median log reduction of 1.255 (range 0.975-4.02). In the combination arm, the median number of BCR-ABL transcripts at diagnosis was 152496 (range 2713-12122052), which had reduced to 15415 (range 4-43524864) at follow-up, a median log reduction of 1.305 (range 0.12-8.2). This reduction in BCR-ABL transcripts in the two groups was not statistically significant (p=0.945). 2 patients in each group achieved more than 3 log reduction in BCR-ABL transcripts. Conclusion: We conclude that addition of cytarabine does not improve significantly upon the molecular responses seen with imatinib alone.

1149 CLINICAL SIGNIFICANCE OF QUANTITATIVE REAL-TIME PCR FOR MONITORING OF MINIMAL RESIDUAL DISEASE FOR PATIENTS WITH CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE RECEIVING GLIVEC THERAPY

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Background. With the high possibility of receiving major cytogenetic response (MCR) and complete cytogenetic response (CCR) for patients with chronic myeloid leukemia (CML) receiving Glivec therapy it is more significant to study minimal residual disease with the help of more sensitive methods than standart cytogenetic analyses. Real-time PCR is a specific and suitable method for quantitative characteristic of molecular response for CML patients treated with Glivec. Aim. Search of prognostically significant levels of minimal residual disease for CML patients in chronic phase (CP) treated with Glivec. Patients and methods. We have analysed 105 samples of peripheral blood and bone marrow and estimated a molecular response (MR) of 59 CML CP patients with MCR and CCR receiving Glivec therapy 400 mg daily after interphases treatment failure. Median of observation was 36 months (6-54 months). We analysed levels of BCR-ABL™ 210 transcript by quantitative Real-time PCR, TaqMan technology (ICycler IQ). β2 microglobuline was used as housekeeping gene. Results were expressed as ratio BCR-ABL/β2 microglobuline x10. We also assessed the lg difference of baseline and the results. The baseline in our investigation has been established according to the analyses of diagnostic levels of BCR-ABL transcript of 41 patients. The baseline level was 33700 BCR-ABL/β2 microglobuline x10. Results. In our investigation we observed a correlation between cytophenic and molecular results, also a correlation between BCR-ABL transcript levels for peripheral blood and bone marrow. In majority (52% of 105) samples residual disease was detected by quantitative Real-time PCR. Decreasing of BCR-ABL transcript levels less than 2 lg from baseline was associated with greater probability of cytogenetic relapse. 3 lg and more decreasing of BCR-ABL transcript levels from baseline was associated with continuous MCR and CCR for all the patients. The patients with cytogenetic relapse had greater median of BCR-ABL transcript level than the patients with MCR and CCR. Cytogenetic relapse was preceded by increasing of BCR-ABL transcript levels. Conclusion. For the majority of CML CP patients treated by Glivec it was possible to detect minimal residual disease with the help of quantitative Real-time PCR. Probability of cytogenetic relapse depends upon BCR-ABL transcript level: less group 2 lg decreasing from baseline in our investigation predicted greater probability of cytogenetic relapse. Real-time PCR should be used as routine analyses for CML patients observation as routine analyses of minimal residual disease for CML patients.

1150 ACUTE HEPATITIS AFTER IMATINIB MESYLATE TREATMENT FOR CML: A CASE REPORT AND REVIEW OF THE LITERATURE

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Imatinib is a well tolerated oral anticancer drug. It is mainly metabolised by liver. Severe hepatic dysfunction occurs in fewer than 5% patients treated. We report a case of severe hepatitis documented by liver biopsy and we analysed similar cases reported in the literature. Case report. A 69-year-old female was diagnosed having CML in the chronic phase. Soon after diagnosis, on 1st August 2003 she started imatinib at a standard dose of 400 mg/d. Three months later she achieved a partial cytogenetic response in the bone marrow and she presented with a slight increase in aspartate aminotransferase (AST 68 U/L) and alanine aminotransferase (ALT 85 U/L). On December 4th imatinib was discontinued because of a progressive increase of hepatic enzymes (AST 158 U/L, ALT 267 U/L). The patient was well and asymptomatic. No other biochemical abnormality was observed. On January 14th, 2004 transaminases peaked at AST 403 U/L and ALT 797 U/L. Serologic tests for hepatitis A, B and C, for EBV, CMV and HSV were all negative. Ultra- sonography of the abdomen was normal. Six weeks after imatinib withdrawal we performed a percutaneous liver biopsy. Histological examination revealed a severe necrosis of hepatocytes with some grade of fibrosis and diffusive inflammatory infiltrates.

Table 1. Clinical and laboratory features.

<table>
<thead>
<tr>
<th>Clinical feature</th>
<th>Imatinib</th>
<th>Other drugs</th>
<th>AST/ALT</th>
<th>Rechallenge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onset of toxicity</td>
<td>12 weeks</td>
<td>10 days</td>
<td>11 days</td>
<td>yes</td>
</tr>
<tr>
<td>Imatinib dose (mg/d)</td>
<td>400</td>
<td>400</td>
<td>250</td>
<td>400</td>
</tr>
<tr>
<td>Other drugs</td>
<td>yes</td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>AST/ALT (U/L)</td>
<td>403/197</td>
<td>220/342</td>
<td>3230/2430</td>
<td>487/159</td>
</tr>
<tr>
<td>Rechallenge</td>
<td>yes</td>
<td>no</td>
<td>yes</td>
<td>no</td>
</tr>
</tbody>
</table>

Pharmacokinetics

<table>
<thead>
<tr>
<th>Pharmacokinetics</th>
<th>Imatinib metabolite</th>
<th>Plasma level</th>
<th>follow-up</th>
<th>AST/ALT at</th>
<th>1.5x</th>
<th>AST/ALT at</th>
<th>1.5x</th>
<th>significant level 617 days after stopping drug</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>not performed</td>
<td>performed</td>
<td>normalized</td>
<td>performed</td>
<td>not performed</td>
<td>performed</td>
<td>not performed</td>
<td>not performed</td>
</tr>
</tbody>
</table>

$*$ Time after discontinuation of imatinib and biopsy; & on imatinib treatment with prednisolone and ursodeoxycholic acid.

On March 2004 bone marrow aspiration documented lost of the cyto- genetic response (100% Ph positive metaphases). Histological examination of liver one year after stopping imatinib was greatly ameliorated and showed minimal changes. We decided to reintroduce the drug (100 mg/kg/day). Plasma levels of imatinib were measured by HPLC/MS on day 1, 10 and 14 (baseline, half-an-hour, 1h, 2h, 4h and 8h after administra- tion). The pharmacokinetics profile of imatinib was comparable to that obtained with standard dose in CML and GIST patients but on day 10 at steady state (SS), a marked increase of the main circulating metabo-
N-desmethyl-imatinib was observed. AUCs (ng/ml h) were 10.2 and 6.3 for imatinib and the metabolite, respectively. Imatinib was stopped from day 11 as transaminases increased (AST 84 U/l, ALT 97 U/l). On day 14 the metabolite plasma level was 86 ng/ml, 3 fold higher than the imatinib concentration. Summary results of the literature. The other cases of imatinib induced hepatotoxicity reported in the literature are described in all cases the patients had no prior evidence of a hepatitis-inducible virus and the liver biopsy showed cytolytic hepatitis of various degree. In one case there was a clear contribution to toxicity of another drug (roxithromycin). Time to onset of hepatic toxicity was quite variable, from a few days to several months of treatment. Interest ingly all the patients were females. Transaminases levels usually normalized after interruption of imatinib. All the patients who had had a drug rechallenge had further hepatic dysfunction. In one case it was possible to continue treatment in association with a corticosteroid. The relatively high level of imatinib N-desmethyl metabolite in plasma of our patient suggests that imatinib metabolism might be involved in the observed hepatic toxicity. Other studies are needed to elucidate this point.

1151. NEW GENOMIC ISSUES ON DER(9) DELETIONS IN CHRONIC MYELOID LEUKEMIA

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Background. The Philadelphia (Ph) chromosome is found in more than 90% of chronic myeloid leukemia (CML) patients. Deletions adjacent to the translocation junction on the derivative chromosome 9 were described by several groups. These studies revealed two main points: 1) genomic microdeletions were concomitant to the t(9;22) translocation; 2) deleted sequences were located upstream to ABL and downstream to BCR genes. We report a detailed molecular cytogenetic characterization of chromosomal rearrangements in two CML cases bearing deletions on der(9) without the characteristics reported above. Aim. We performed a molecular cytogenetic analysis by FISH to precisely characterize chromosomal events occurring in a case bearing a complex variant t(9;22) and in a case with ins(9;22)(q34;q11). Methods. Both patients were diagnosed and tested by conventional cytogenetic analysis, fluorescence in situ hybridization (FISH), and RT-PCR. FISH identification of the ABL and BCR genes was performed using a pool of PAC, RPS-85322 and RPS-112812, and the BAC RP11-164N13, respectively. A set of BAC/PAC probes (proximal and distal to ABL and BCR, respectively) belonging to 9 and 22 chromosomes allowed us to define precisely the deletion size. The UCSC database (http://www.genome.ucsc.edu) was queried for BAC/PAC probe locations and for gene identification. Results. Case #1. FISH experiment with BCR and ABL specific probes revealed one fusion signal on der(22) chromosome, a faint ABL signal on der(9) and a split BCR signal on der(6) and on der(12). Retention of BCR specific signals was confirmed after hybridization with ABL/PAC clones, allowed the precise definition of the complex rearrangement breakpoints. Surprisingly, the detailed molecular cytogenetic characterization of chromosome 9 breakpoint showed genomic loss of about 400 Kb downstream to ABL gene. NUP144 is the alone gene with known function mapping in the deleted region. According to our FISH results, the revised karyotype was the following 46.XX,t(6;9;12;22)(p22;q22;q34;q11). Case #2. Conventional cytogenetic analysis revealed a normal karyotype. FISH analysis with clones specific for ABL and BCR genes showed a single fusion signal on der(9). These results suggested the occurrence of a cryptic insertion generating a 5' BCR/5' ABL fusion gene on the der(9) instead of 22q11. Further FISH experiments using clones located proximally to BCR showed that a chromosome 22 region of 3 Mb was inserted on 9q34. The use of BAC clones proximal to ABL and distal to BCR showed the loss of chromosomes 9 and 22 sequences on der(9). Two known and one candidate tumor suppressor genes (TSGs) map in the deleted regions: SMARCB1 (SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin subfamily B member 1) and GSTT1 (glutathione S-transferase t) in 22q11, and PRDM12 (PR domain containing 12) in 9q34. Conclusions. Our data indicate that deletions on der(9) in CML cases could also involve chromosomes 9 sequences located telomeric to the ABL gene apart from the centromeric sequences previously described. Moreover, genomic microdeletions can be associated to rearrangements involving 9 and 22 chromosomes, such as insertion event, other than reciprocal translocation.
sibility of intensive chemotherapy in these patients. The aim of the study is to value the difference in EFS and OS among 2 groups of AML elderly patients treated with intensive chemotherapy (IC) or maintenance (M). From June 2001 to January 2006 we have treated in our Division 54 AML patients, 30 male and 24 female with median age of 73 years (66-90 years). 27 patients (16 M and 11 F with median age of 71 years) have received intensive chemotherapy (I.C. Flag and MICE) and 27 (14 M and 13 F with median age of 78.5 years) have received maintenance (low dose cytarabine and/or support). In IC group 12 patients (45%) have obtained to complete remission (CR) with to EFS and OS media of 4,47 and 7,15 months respectively, the rate of TRM has been of 25%. In the M group the CR has been documented in 8 patients (50%) with to EFS and OS media of 4,22 and 4,94 months respectively (graph 1).

This results have shown a best rate of CR in the IC group but the OS and EFS difference is not statistically significant in the two groups (p=0.7). In conclusion the Intensive chemotherapy has not improved the survival in AML elderly patients. New therapeutics strategy is necessary for to improve the EFS and OS in these patients. Interesting is the use of specific monoclonal antibodies (anti CD33) in this poor disease especially in maintenance after a CR obtainable with an intensive or low dose chemotherapy.

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ACUTE MYELOID LEUKEMIA IN PATIENTS AGED 70 OR OLDER. EXPERIENCE AT A SINGLE CENTRE
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The management of old patients with acute myeloid leukemia remains controversial, specially in those cases that can be considered very old patients (aged 70 or older) in which the dilemma therapeutic abstention versus treatment (with low or high intensity) can be considered. We present our experience with this group of patients in the period 1990-2005. During the period of study 56 cases were diagnosed (FAB M3 cases were excluded). Patients were divided into 3 groups according to the treatment: no treatment, low intensity treatment (low doses Ara-C: 10 mg/m2 s.c. days 1-21) and high intensity treatment (adapted ICE: Idarubicin 10 mg/m2 days 1 and 3; Ara-C 100 mg/m2/12h days 1-3; Etoposide 100 mg/m2/72h days 1-3). The mean age of patients was 76.29 years (70-85); sex distribution was 29 males and 27 females; mean Karnofsky index was 70.5 (30-100); 39 patients received treatment and 17 did not; overall survival was 5.8 months (median 2; 0.06-90+), almost significant differences were observed in the mean overall survival between the treated and no-treated groups (4.4 vs 2.1 months respectively; p=0.06). In the low intensity group (30 patients) an overall response of 53.3% (6 CR, 4 PR, 13 NR and 7 not evaluable) was observed while in the high intensity one (9 patients) this overall response was 44.4% (4 CR, 0 PR, 3 NR and 2 not evaluable); no statistical differences were observed between these two groups (p=0.16). Considering overall survival in these same groups, no statistical differences were observed between them 7.2 (0.25-90+) vs 8.1 (0.5-28+) months (p=0.95) respectively between the low and high intensity groups. Overall survival in the treated group is higher than in the non-treated one, differences almost reach statistical significance (p=0.06). Though no statistical differences have been observed in the overall survival between both groups of treatment, this event could be explained by two reasons: the very long survival in one patient in the low intensity group and the still short follow-up of some patients in the high intensity one. Comparing both arms of treatment, a higher proportion of CR can be observed in the high intensity group (44.4% vs 20%, respectively), however, if this circumstance will contribute to a longer survival is still unknown.

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NKGU RECEPTOR EXPRESSIONS IN UNTREATED ADULT PATIENTS WITH ACUTE MYELOGENOUS LEUKEMIA
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Backgrounds. Natural killer (NK) cell is one of the important cytotoxic lymphocytes for innate immune response to tumor cells as well as infected cells. The balance of the activating and inhibitory receptors of NK cells can determine the activity of cytotoxicity. Based on recent advances in the understanding of NK cells, baseline expressions of NKG2K+ and CD94+ in adult patients with AML. Aims. In this study, we investigated expressions of C-type lectin-like receptors, i.e. CD94/NKG2A and NKG2D, in adult patients with AML. Together, it is expected that other researchers will also examine the role of ethnic differences in the phenomena described here. Methods. PB samples from 24 normal donors and 14 untreated AML patients were enrolled. Flow cytometry analysis using CD56, CD16, CD3, NKG2A, and NKG2D-specific monoclonal antibodies were performed. Results. The proportion of CD16+CD3- NK cell and CD56+CD11b- NK cell in peripheral lymphocytes were 3.4±3.8% vs 8.2±3.5% and 4.0±2.7% vs 8.6±1.7% in AML and control, respectively. NKG2D+ and CD94/NKG2A+ cells among CD56+CD11b- fraction were 35.7±5.4% vs 24±3.9% (p=0.13) and 26.1±5.4% vs 87±7.7% (p=0.04), respectively. Therefore, NKG2D expression of NK cells in AML patients was statistically significantly decreased compare to control (p=0.050). Summary/Conclusions. While the expression of NKG2D, activating receptor of NK cell is relatively increased, the expression of CD94/NKG2A, inhibitory receptor of NK cell is decreased in AML patients, which means some other mechanisms including altered responding ligand(s) are engaged.

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INCREASE IN SERUM SOLUBLE HLA-I IN ACUTE MYELOGENOUS LEUKEMIAS LEADS TO FAS LIGAND-MEDIATED APOPTOSIS OF CD8+ LYMPHOCYTES
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Soluble HLA-I (shLA-I) molecules have been firstly described in the serum and urine of healthy individuals. More recently, it has been shown that the serum level of these soluble molecules is significantly increased in patients with an activation of their immune system, such as during allograft rejection, acute graft versus host disease after bone marrow transplantation, autoimmune diseases or viral infections. Moreover, shLA-I molecules can be released by tumor cells, and high shLA-I serum levels have been found in solid cancers, melanomas and lymphomas. Thus, shLA-I molecule are not specific markers for organ rejection, but rather are affected by inflammatory processes and viral or neoplastic transformation. In this study, we show that high serum levels of soluble HLA class I molecules (shLA-I, range: 0.7-1.7 mg/mL) and soluble Fas ligand (Fasl, range: 0.4-1.9 ng/mL) are detected in patients with acute myeloid leukemia (AML) at diagnosis, compared to healthy donors (shLA-I range: 0.1-0.6 mg/mL; sFasL range: 0.1-0.4 ng/mL). Both shLA-I and sFasl serum concentrations increased during chemotherapy. The functional role of shLA-I molecules either in physiological or in pathological conditions is not clear: it has been described that HLA-I molecules can trigger cytotoxic T lymphocytes to release cytolytic enzymes and pro-inflammatory cytokines. However, we and others reported that shLA-I molecules bind to CD8 receptors expressed on cytotoxic effector lymphocytes leading to activation-induced apoptosis or cell death mediated by synthesis and secretion of Fasl, and the consequent inter-
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action with Fas expressed by T and NK cells. AML patients’ sera were
able to induce transcription and secretion of FasL in CD8+ T cells, followed by apoptosis in vitro; this apoptosis was inhibited by either antiHLA-I or anti-FasL specific monoclonal antibodies. These findings closely relate to the in vivo up-regulation of FasL transcription observed in
peripheral blood lymphocytes from AML patients; in the same cells,
mRNA for the antiapoptotic protein Bcl-2 was down-regulated. Interestingly, caspase-8 and caspase-3, both downstream mediators of death
receptors-induced apoptosis, were activated in vivo in CD8+ cells of AML
patients, but not of healthy donors. These data strongly suggest that in
AML, increased levels of sHLA-I molecules may be responsible for the
elimination of potentially anti-tumor effector cells through a FasL/Fas
interaction.

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THE NUMBER AND APOPTOSIS OF CIRCULATING ENDOTHELIAL CELLS IN THE
PERIPHERAL BLOOD IS SIGNIFICANTLY INCREASED IN PATIENTS WITH ACUTE MYELOID
LEUKEMIA AND REFRACTORY ANEMIA WITH EXCESS OF BLASTS
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Objectives. The circulating endothelial cells (CEC) are proposed to be
a noninvasive marker of angiogenesis. Material and Methods. We evaluated the absolute counts of CEC, their resting (rCEC) and activated
(aCEC) subsets, circulating endothelial progenitor cells (CEPC) as well
as apoptotic CEC (CECAnnV+) in peripheral blood (PB) of 70 untreated
patients with acute myeloid leukemia (AML) and 23 with myelodysplastic syndrome RAEB type (RAEB). The control group consisted of 30
healthy controls. CEC counts were evaluated by four-colour flow cytometry using a previously described panel of monoclonal antibodies. CEPC
were defined as CD45–, CD34+, CD31+ and CD133+. rCEC were defined
as CD45–, CD133–, CD31+, CD34+, CD146+ and negative for activation
markers (CD105, CD106). CD105 or CD106 positive mature endothelial cells were classified as aCEC. The levels of CEC were correlated
with known prognostic factors. Additionally, apoptotic CEC were
detected in PB using Annexin V assay (CD146+/Annexin-V+ cells,
CECAnnV+). The percentage of CECAnnV+ among the whole CEC number was determined. Results. There were highly significant differences in
the count of CEC and their particular subtypes between AML and RAEB
patients as well as the controls. The results (median counts and ranges)
are presented in the Table. The positive correlation between CEC and
CEPC counts was observed in both AML (r=0,435; p<0,001) and RAEB
(r=0,634; p<0,01). The numbers of apoptotic CEC (CECAnnV+) in both
AML and RAEB were significantly higher than in the control (p<0,0001).
However, in patients with RAEB the rate of CECAnnV+ was significantly higher than in those with AML (p<0,0001). The number of
microvascular origin CEC, depicted by CD36 expression, was also higher in MDS than in both AML (p<0,0001). Moreover, the negative correlation between CEC and absolute counts of white blood cells as well as
PB blasts was observed in RAEB but not in AML. Conclusions. The CEC
levels are significantly higher in AML and RAEB patients than in healthy
subjects. These findings may suggest a relationship between clonal transformation and the substantially increased number of CEC. The rate of
CECAnnV+ is significantly elevated in AML and RAEB what may be due
to increased turnover of CEC. Distinctly lower propensity of CEC to
undergo apoptosis found in AML may correspond with more aggressive
clinical course of this disease.
Type of

AML

MDS-RAEB

Control group

endothelial
cells

n=70
(a)

n=23
(b)

n=30
(c)

CEC (/µL)

27,2
(3,9-291,3)

12,5
(4-39,7)

a CEC (/µL)

11,35
(0-87,7)

5,4
(1,6-33,1)

r CEC (/µL)

11,85
(0-203,6)

7,8
(1,2-21)

CEPC (/µL)

2,25
(0-40,2)

1,9
(0-12,2)

p value

2,95
a vs. c p<0,0001
(0,5-13,1) b vs. c p<0,0001
a vs. b p<0,0001
0,9
a vs. c p<0,0001
(0-5,2)
b vs. c p<0,0001
a vs. b p<0,01
1,6
a vs. c p<0,0001
(0,4-10,68) b vs. c p<0,0001
a vs. b p<0,03
0,1
a vs. c p<0,0001
(0-1,2)
b vs. c p<0,001
a vs. b p<n.s.

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AN ANTECEDENT DIAGNOSIS OF REFRACTORY ANEMIA WITH BLAST EXCESS HAS NO
PROGNOSTIC RELEVANCE IN ACUTE MYELOID LEUKEMIA OF THE ELDERLY TREATED
WITH AGGRESSIVE CHEMOTHERAPY
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Background. Host related factors and disease related factors account for
the unsatisfactory outcome of acute myeloid leukemia (AML) in the elderly. However, the exclusion in many trials of patients with previously
diagnosed myelodysplastic syndrome (MDS) renders uncertain the evaluation of the prognostic relevance of secondary AML (s-AML), defined
as AML arising after either a history of chemotherapy or radiotherapy
for a previous malignancy or a preceding history of MDS or hematologic malignancies. Aims. To evaluate the prognostic relevance of a previous
diagnosis of refractory anemia with excess of blasts (RAEB) in terms of
complete remission (CR) achievement and duration, survival and feasibility of autologous stem cell transplantation (ASCT) in elderly patients
with AML. Patients and methods. Among 166 consecutive elderly AML
patients observed in the period 2001-2005, 87 cases (median age: 69
years, range 61-81) were enrolled into an aggressive chemotherapy program consisting of a combination of fludarabine and intermediate dose
cytarabine given as continuous infusion (c.i.) [fludarabine: loading dose
of 10 mg/m2 at day 0 followed by a c.i. of 20 mg/m2/24h for 72h; cytarabine: loading dose of 390 mg/m2 (infusion duration: 3h) 3.5h after fludarabine and then as c.i. at 1440 mg/m2/24h for a total of 96h]. Fourtynine patients (56%) were diagnosed as having de novo AML, while a
diagnosis of s-AML was made in 38 cases (44%). Cytogenetic analysis
(76 cases evaluable), showed a normal karyotype in 45 patients (59%),
complex karyotype or other unfavorable abnormalities in 31 (41%). No
basal characteristic (age, WBC at diagnosis, FAB, cytogenetics) was statistically different between the two groups. A conditioning regimen consisting of 2 days c.i. idarubicin (20 mg/m2)and 3 days oral busulphan (4
mg/kg) was used. Results. Overall, 56 patients (64%) achieved CR, 29
(33%) were able to mobilize a sufficient number of peripheral blood
stem cells, and 23 (26%) were actually autografted. CR rate (61% vs
68%, p:0.63), death in induction (20% vs 16%, p:0.78) and primary
resistance (18% vs 16%, p=0.97) were no statistically different between
the group of de novo and s.AML. Median time for neutrophil recovery
was similar, while s-AML patients required longer time for platelet recovery (p=0.04). There was no difference as to eligibility for consolidation
(87% vs 77%, p:0.54) as well as for mobilization and feasibility of ASCT
(16/20 vs 13/18, p=0.85, and 14/16 vs 9/13, p=0.52, respectively). s-AML
had negligible impact on overall survival (OS) and disease free survival
(DFS), (OS: 8 vs 8 months, p=0.53, DFS: 10 vs 9 months, p=0.41). In the
multivariate analysis the only parameter significantly related to either OS
or DFS duration was adverse karyotype (p=0.02 and 0.04, respectively).
Conclusions. A diagnosis of s-AML does not represent a clinically relevant
prognostic factor in elderly AML patients treated with aggressive therapy in our series. Furthermore, s-AML patients can be mobilized and
autografted with comparable results as opposed to de novo cases.

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SERUM CONCENTRATION OF SOLUBLE E-CADHERIN (SE-CADHERIN) AND β-CATENIN
EXPRESSION IN LEUKEMIC CELLS IN ACUTE MYELOBLASTIC LEUKEMIA (AML) PATIENTS
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Background. A tumour transformation is associated with disorders of
intracellular structures function, participated in adhesion-dependent signalization, such as: E’cadherin and β-catenin.E-cadherin is a transmembrane glycoprotein that mediates intercellular adhesion. Its soluble form
(sE-cadherin) was found in biological fluids of healthy persons. Serum
concentration of sE-cadherin are elevated in patients with malignancies
of epithelial origin. β-catenin is a multifunctional protein, which plays a
role as a component of the cell-cell adhesion apparatus. The function for
β-catenin in hematological malignancies has not been reported. Overexpression of β-catenin has been found in Jurcat cells, K562 cell line and
HUT-102 cells. Reduction of β-catenin nuclear signalling inhibited proliferation and clonogenicity in these cell lines. The data suggest that ßcatenin can play a significant role in promoting leukemic cell proliferation, adhesion and survival. AIM. Aims of our study were: soluble E’cadhaematologica/the hematology journal | 2006; 91(s1) | 421


herin (sE-cadherin) serum concentrations comparison in acute myeloid leukemia patients (AML) and in controls and estimation of sE-cadherin serum concentration and β-catenin expression in leukemic cells of AML patients at the time of diagnosis. Patients and methods. Forty-eight patients were included: 21 men and 27 women aged 20-79 years (x±s:42). According to FAB classification: 1 patient with M0, 10 with M1, 10 with M2, 1 with M3, 15 with M4, 6 with M5, 6 with M6 and 1 patient with biphenotypic leukemia. Sixteen patients reached complete remission (CR). Results. We have suggested that sE-cadherin serum concentration and β-catenin expression in leukemic cells were statistically higher in AML patients with primary resistance to chemotherapy than in AML patients with complete remission (61.9±22.05 vs 57.9±22.6 ng/l, p<0.05). The correlation for sE-cadherin serum concentration was 0.6% vs 1.4% (r:0.038), p<0.000 for β-catenin expression). We have also indicated the positive correlation between sE-cadherin serum concentration and β-catenin expression in leukemic cells in AML patients and between patients age and sE-cadherin serum concentration. Summary. Our data indicate that sE-cadherin serum concentration and β-catenin expression in leukemic cells could be considered as additional prognostic markers in AML.

A NEW HUMAN ACUTE MONOCYTIC LEUKEMIA CELL LINE TZ-1 WITH T(1;11)(P32;Q23) AND LYMPHOID PHENOTYPES

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Background. Human leukemia cell lines are of great value in investigating basic and applied aspects of cell biology and clinical medicine. Leukemia cell lines have been instrumental in the biological and molecular analysis of recurring chromosome rearrangements, notably translocations. In addition, these cell lines have contributed to a better understanding of the pathogenesis of human leukemias. Translocations targeting the MLL gene at 11q23 have come to represent a paradigm in acute leukemia. It has been reported that there are more than 80 partner genes for MLL. Although the functions of fusion transcripts remain large-standing of the pathogenesis of human leukemias. Translocations targeting the MLL gene at 11q23 and the MLL-AF1 fusion transcript. Taken together, TZ-1 is a promising model in the study for analyzing the pathogenesis of MLL-AF1-positive leukemia and developing new agents for this type of leukemia.

DIFFERENTIATION OF HUMAN ACUTE MYELOID LEUKEMIA CELLS IN PRIMARY CULTURE IN RESPONSE TO DERIVATIVES OF METHYL ASOMONATE, PLANT STRESS STRESS HORMONE

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Backgrounds. Since several plant hormones and their analogs induce cell cycle arrest and inhibit human cell proliferation, these compounds may be therapeutic agents against human malignancies. Some regulators of plant growth and differentiation have been shown to induce the differentiation of several human myeloid leukemia cells, and might be effective as differentiation inducers to control acute myeloid leukemia (AML) cells. Methods. Several myeloid leukemia cell lines were cultured with methyl asomonate (MJ) and its derivatives. Cell differentiation was determined by nitroblue tetrazolium-reducing activity, morphological changes, α-naphthyl acetate esterase activity and expression of differentiation-associated surface antigens. Results. MJ induced both monocytic and granulocytic differentiation of HL-60 cells. MJ activated mitogen-activated protein kinase (MAPK) in the cells before causing myelomonocytic differentiation. MAPK activation was necessary for MJ-induced differentiation, since PD98059, an inhibitor of MAPK kinase, suppressed the differentiation induced by MJ. This new finding improves our understanding of other human leukemia cell lines. Introduction of a double bond at the 4,5-position greatly enhanced the differentiation-inducing activity of MJ. Although differentiation-inducers potently affect the differentiation of AML, the role of cell lines other than HL-60 cells in their activity remains unexplored.

CD56 ANTIGEN EXPRESSION IN ACUTE MYELOID LEUKEMIA: CLINICAL AND IMMUNOPHENOTYPIC IMPLICATIONS

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Background and Aims. The CD56 antigen expression has been reported in several hematologic malignancies; it is found in 10-30% of cases of acute myeloid leukemia (AML). Its prognostic impact remains uncertain although it has been associated with an unfavorable outcome, especially in AML M4 and M5 subtypes. Recently, two novel CD56+ malignancies (the CD7-/CD56+ myeloid/NK and the CD7+CD56+ dendritic cell malignancies) were described. We investigated the immunophenotypic identity of the CD56+ AML and its correlations with other disease characteristics. Patients and Methods. From 1999 to 2005, 127 samples of fresh bone marrow or peripheral blood of AML patients were analyzed in our laboratory. One-, two- or three-color flow cytometry was performed on Coulter Epics cytometer. Positivity for surface sE-cadherin serum concentration was set at 20% and for cytoplasmic (c) antigens at 10% of blasts stained with specific monoclonal antibodies for glycoporphin A, CD2, sCD5, CD4, CD7, CD8, cCD13, CD14, cCD19, sCD20, cCD22, CD3, CD45, CD61, CD117, HLA-DR, MPO, lysozyme, lactoferrin, CD79a, CD8, cCD22, CD13 and TdT. Results. Patients (pts) were classified according to FAB criteria as M0:10 pts, M1:25, M2:20, M4:31, M5:12, M6:1 and M7:2 pts. 15.7% of the pts suffered from secondary leukemia. CD56 expression was detected in 29% (35/127) of the pts; this was higher in M1/2 (33%) and M5 subtypes (52%). CD56 positivity was not influenced by age, sex, WBC, blast percentage, Hb and platelet count at diagnosis, LDH, secondary leukemia and extramedullary disease. Statistical analysis revealed a positive correlation between CD56 expression and expression of CD3 (0.001, r:0.329), CD2 (p:0.014, r:0.221), CD3 (p:0.047, r:0.298) and CD2 (p:0.017, r:0.281) antigens, while a negative correlation was detected for sCD13 (r:0.038, r:0.187) and cCD13 (p:0.081, r:0.163) expression. CD56+ AML group expressed also CD38 (79% of the pts), lysozyme (79%), HLA-DR (78%), cCD13 (71.4%), MPO (69%) and CD61 (67%). The comparison between the CD56+ AML group and the CD56- leukemias showed differences in the expression of lysozyme (79% of CD56+ AML vs. 54%, p<0.044), CD22 (6% vs. 0%, p:0.03), CD34 (67% vs. 92%, p:0.071) and CD13 (55% vs. 71%, p:0.066). Conclusions. The immunophenotype of CD56+ AML is characterized by the commitment to the myeloid/monocytic lineage (CD58, lysozyme, cCD13, HLADR and MPO). The negative correlation between CD56 expression and CD13 and cCD13 is a novel finding not previously reported. The further investigation of the possible relationship of the CD56+ AML with the NK/dendritic cell leukemias might be the key to explain its clinical behavior.

DIFFERENTIATION OF HUMAN ACUTE MYELOID LEUKEMIA CELLS IN PRIMARY CULTURE IN RESPONSE TO DERIVATIVES OF METHYL ASOMONATE, PLANT STRESS STRESS HORMONE

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of established cell lines, they may have only modest differentiation-inducing activity in freshly isolated leukemic cells. Therefore, we sought to determine whether the potent derivative of MJ could affect the differentiation of leukemic cells from patients with AML. In the present study, we examined the effect of MJ derivative on the differentiation of AML cells in primary culture and compared this differentiation-inducing activity with those of the well-known inducers all-trans retinoic acid and 1α,25-dihydroxyvitamin D3. Jasmonate derivatives significantly stimulated both functional and morphological differentiation of leukemia cells in some AML cases. This differentiation-inducing activity was more potent than those of all-trans retinoic acid and 1α,25-dihydroxyvitamin D3. Jasmonate derivatives significantly stimulated both functional and morphological differentiation of leukemia cells in some AML cases. This differentiation-inducing activity was more potent than those of all-trans retinoic acid and 1α,25-dihydroxyvitamin D3. Jasmonate derivatives significantly stimulated both functional and morphological differentiation of leukemia cells in some AML cases. This differentiation-inducing activity was more potent than those of all-trans retinoic acid and 1α,25-dihydroxyvitamin D3. Jasmonate derivatives significantly stimulated both functional and morphological differentiation of leukemia cells in some AML cases. This differentiation-inducing activity was more potent than those of all-trans retinoic acid and 1α,25-dihydroxyvitamin D3. Jasmonate derivatives significantly stimulated both functional and morphological differentiation of leukemia cells in some AML cases.

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**FLAG-(IDA)-MYLOTARG IN THE THERAPY OF RELAPSED AND REFRACTORY ACUTE MYELOID LEUKEMIA. PRELIMINARY RESULTS**

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**Background.** Patients with relapsed and refractory AML have a bad prognosis. In this setting it is often difficult to obtain a prolonged complete remission. In the last years Fludarabine and high dose Cytarabine with or without Idarubicine and G-CSF have been frequently used (FLAG and IDA schedules). The immunotoxin gemtuzumab ozogamicin (Mylotarg)(GO) is a humanized IgG4 monoclonal antibody directed against the CD33 epitope, which is chemically linked to calicheamicin. Fludarabine and Ara-C with or without Idarubicine and G-CSF are the most well accepted regimen in the treatment of relapsed AML patients. FLAG-(IDA)-GO schedule was in our experience a feasible therapy with acceptable toxicity. The positive clones were selected, and further characterization of these compounds may be clinically useful. One novel derivative is a particularly promising therapeutic agent for the treatment of leukemia.

**1165**

**FLAG-IDA IN THE TREATMENT OF REFRACTORY /RELAPSED ADULT ACUTE MYELOID LEUKEMIA**


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**Background.** Although several different chemotherapy combinations have been administered to patients with refractory/relapsed acute myeloid leukemia (AML), the prognosis in this subset of patients is still poor, with a complete remission (CR) rate ranging from 30 to 40%. The goal of reinduction chemotherapy varies from achievement of long-term CR to providing a bridge to hematopoietic stem cell transplantation (HSCT) aimed at prolonging disease-free (DFS) and overall survival (OS). **Aims.** In this study we evaluated the efficacy and the toxicity profiles of FLAG-IDA (Fludarabine 30 mg/m², Ara-C 2 gr/m² for 5 days, idarubicin 10 mg/m² for 3 days and G-CSF 5 mg/kg per day until neutrophil recovery). All patients underwent cytogenetic evaluation: 4 (5.4%) were resistant in the favourable-risk group, 31 (41.8%) in the poor-risk group and in 9 (12.2%) the karyotype was not available. Fifty-four patients (72.9%) were in first relapse; 44 after only chemotherapy, 7 after chemotherapy and autologous peripheral stem cell transplantation and 3 after chemotherapy and allogeneic peripheral stem cell transplantation. Twenty patients (27.1%) were refractory to conventional chemotherapy including cytarabine, etoposide and daunorubicin. **Results.** The overall CR rate was 48.6% (36 of 74); 28 of 54 (51.8%) in relapsed and 8 of 20 (40%) in refractory patients. There were 5/74 deaths (6.7%), 1 due to fungemia (C. tropicalis), 2 to sepsis (P. aeruginosa) and 2 to cerebral hemorrhage. 35 of 74 patients (44.5%) were resistant to FLAG-IDA. All patients experienced profound neutropenia (<0.1×10⁹/L); in patients achieving remission the median time to reach PMN>0.5×10⁹/L and 1×10⁹/L was 20 (range: 16-27) and 23 (range: 17-30) days; median time to achieve platelet levels >20×10⁹/L and 4×10⁹/L was 28 (range: 17-58) and 31 days respectively. During the neutropenic phase, 25 episodes of documented sepsis (33.7%) were observed. Febrile neutropenia lasted a median of 7 days; seven patients had no fever at all. As to non hematological toxicity, the most common side effects were mucositis (60 of 74 or 81.1%) and an increase of serum bilirubin (25 of 74 or 33.7%). After achieving CR, 21 patients received allogeneic stem cell transplantation (11 from a matched donor, 5 from a mismatched donor and 5 from an unrelated donor) and 4 patients received autologous stem cell cell transplantation; 6 patients were judged unable to receive any further therapy and 5 refused other therapy. In the 36 responders, the disease-free survival (DFS) and overall survival (OS) and 12 relapses and 4 deaths, respectively; the 21 patients who received allogeneic stem cell transplantation had a DFS of 18 (range 4 - 68) months. **Conclusions.** In our experience, FLAG-IDA is a well-tolerated regimen in relapsed/refractory AML patients; the toxicity is acceptable, enabling most patients to receive further treatment, including transplantation procedures.
1167 PREVALENCE AND PROGNOSTIC SIGNIFICANCE OF FLT3 MUTATIONS IN ACUTE MYELOID LEUKEMIA: ASSOCIATION OF ITDS WITH POOR OUTCOME IN PATIENTS WITH NORMAL CYTOGENETICS

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Backgrounds. Acute myeloid leukemia (AML) is a difficult disease to treat, and novel molecular targeted therapy represents a novel therapeutic approach. Activating mutations of FMS-like tyrosine kinase 3 (FLT3) are present in approximately one third of patients with de novo AML and have been implicated in its pathogenesis. The leukemic blasts of most AML patients have the internal tandem duplications (ITDs) in the juxtamembrane region or point mutations in Asp835 and Iso836 of the kinase domain (TKD) of the FLT3 receptor. Both mutations result in constitutive FLT3 receptor activity and may play a significant role in leukemogenesis. Aims. In this study we have analyzed the incidence and type of FLT3 mutations in a large series of newly diagnosed AML patients. Furthermore, we have evaluated the prognostic impact of FLT3 mutations. Methods. The FLT3/ITD was determined by polymerase chain reaction (PCR). The mutations of D835 and I836 codons were determined by PCR followed by restriction enzyme digestion (PCR-RFLP). For the estimation of the statistical significance of the differences in the clinical-biological characteristics, between the mutated patients and wild-type patients, it has been used the Student’s t test for independent data. The probabilities of overall survival (OS) and disease free survival (DFS) were analysed by Kaplan-Meier method; the differences of OS and DFS, between the mutated patients and wild-type patients, were assessed using the log-rank test. Results. Both FLT3/ITD and FLT3/TKD mutations were found in 15%. Dual mutations were found in 2% of 126 patients. Among the FAB subtypes of AML, the rate of FLT3 aberrations was higher in M4 (27%) and M5 (26%). FLT3/ITD was associated to leukocytosis (106.8 x 10⁹/L vs 30 x 10⁹/L in FLT3-wt, p=0.015) and high percentage of circulating blast cells (82% vs 42% in FLT3-wt, p<0.0001). Differently, FLT3/TKD mutations were not associated with high white blood cells count and blast cells percentage. FLT3 mutations were more prevalent in patients with normal karyotype (51%). In this group, DFS and OS were significantly inferior for patients with FLT3/ITD than patients without mutation (0 vs 5, p=0.0052; 5 vs 9, p=0.049, respectively). Conclusions. We have identified the FLT3/ITD as an independent poor prognostic factor in AML patients with normal cytogenetics. Therefore, targeting FLT3 mutations represents a potential therapeutic target for AML. These results suggest that new treatment modalities, such as therapy with a FLT3 tyrosine kinase inhibitor, are clearly needed for this group of patients with “standard risk” profile.

Acknowledgments: COFIN 2005 (Myelodysplastic syndromes: pathogenetic models and promise of new therapies), COFIN 2003 (Molecular therapy of leukemias), FIRB 2001, by the University of Bologna (60%), by the Italian Association for cancer research (A.I.R.C.), by the Italian National Research Council (C.N.R.), by Fondazione Del Monte of Bologna e Ravenna (Italy) and A.I.L. grants.

1168 IMPACT OF CHEMOTHERAPY TREATMENT IN AML PATIENTS AGED MORE THAN 60 YEARS OLD. A SINGLE CENTRE EXPERIENCE

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Introduction: Overall prognosis in elderly patients with acute myeloid leukaemia (AML) is very poor. Nowadays patients older than 60 years who receive conventional chemotherapy achieve complete remission (CR) in <60% and <15% of whom are free of disease more than 3 years. Despite palliative treatment strategies has historically been considered dismal, a comparison between aggressive treatment and non-aggressive approach is needed. Objectives: We retrospectively analyzed the impact on survival of a conventional treatment with chemotherapy versus a symptomatic approach in AML patients aged more than 60 years old in a single centre. Patients and methods: Between January 1974 to September 2005, 409 patients older than 60 years old with a diagnosis of AML were enrolled in our registry, 241 (59.9%) were males and 168 (41.1%) females. The median age of the whole group was 71 years (range 60-95 years). AML subtypes according to FAB classification were: M0 (5.7%), M1 (15.1%), M2 (8.5%), M4 (14.5%), M5 (16.8%), M6 (9.1%), M7 (1.4%) and AML-NOS (28.8%). Survival data were available from 267 patients. According to medical decision, 104 patients (38.9%) received intensive treatment, mainly based on conventional chemotherapy that included an anthracycline with cytarabine. The remaining 163 (61.1%) patients were managed with conservative approaches. Results: Analyzing patients aged > 60 years, the actuarial survival at 10 years in the group that received intensive treatment was 11.2% versus 2.2% for those receiving best supportive care (p<0.001). Considering only patients with de novo AML, actuarial survival at 10 years was also better for patients receiving intensive chemotherapy (14.7% versus 0%; p<0.001). Patients aged > 70 years (n: 157) also benefit of an aggressive approach (actuarial survival at 10 years: 14.5% vs 2.7%; p=0.012), specially when considering patients with de novo AML (n: 105); 10% vs 0% (p=0.001). The group of patients aged between 70-80 years (n: 76) with de novo AML presented an actuarial survival of 10% vs 0% at 10 years (p=0.005). In contraposition to these results, in patients aged > 60 years with AML and previous MDS no significant differences in survival were observed in basis to receive or not treatment with chemotherapy. Conclusions. Our results probe the benefit of treatment in elderly patients with AML mainly in those diagnosed of de novo AML.

1169 ADULT ACUTE MYELOID LEUKEMIA (AML) WITH DEL (16) (q22): A REPORT OF 11 CASES

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1Hospital Edouard Herriot, LYON, France; 2Laboratory of Hematology, LYON, France; 3Leukemia Unit, LYON, France

Background. Cytogenetic analysis of leukemic blasts performed at diagnosis is generally recognized as the single most valuable prognostic factor in AML. Three large collaborative studies (MRC, SWOG/ECOG, CALGB) assigned AML patients to one of the three risk groups (favorable, intermediate or adverse) based on pretreatment cytogenetic findings. There are cytogenetic hallmarks of core-binding factor (CBF) AML, including t(8;21) and chromosome 16 abnormalities, which have been categorized in the favorable group by all three cytogenetic classifications. However, the three cytogenetic risk systems differ with regard to del (16q). SWOG/ECOG classified patients with del (16q) as favorable, while MRC and CALGB did not. Patients and methods. We report 11 adults with newly diagnosed AML presenting with del (16q) with the aim of evaluating cytological and clinical features, and the outcome after therapy. Median age was 68 years (range, 33 - 88). The sex ratio male/female was 1.2. Results. Median hematological features at presentation were as followed: WBC count at 7 x 10⁹/L (1.5 - 469), hemoglobin level at 94 g/l (71 - 154), platelet count at 88 x 10⁹/L. Circulating blasts were present in 9 cases. Six patients presented with M5 AML subtype of whom 1 patient displayed eosinophilia, 3 patients presented with M4 AML subtype of whom 1 patient had eosinophilia. Two cases had AML with multilineage dysplasia. Overall 6 cases presented dysgranulopoiesis features, while 4 of them also displayed dyserythropoiesis. In all cases karyotype at diagnosis presented numerical and/or structural abnormalities combined with del (16)(q22). Only 3 patients achieved complete remission (CR) (27%) after induction chemotherapy. All three patients relapsed: after 2, 3 and 7 months of CR respectively. Median overall survival was 4 months (0 - 30 months). Conclusion. AML patients with true del (16q) are usually found in AML with evidence of myelodysplasia and morphology other than that of acute myelomonocytic leukemia with abnormal eosinophils. They should not be included in the favorable cytogenetic risk group. Consequently, the RT-PCR and/or FISH assays detecting CBFB-MYH11 gene fusion should be performed in all patients with del (16q)(q22) to ensure that they do not harbor a misidentified inv (16) / t (16;16).

424 | haematologica/the hematology journal | 2006; 91(s1)
Acquired activating mutations of both fms-like tyrosine kinase 3 (FLT3) and Janus kinase 2 (JAK2) confer proliferative and survival advantage for leukemic cell clones. Internal tandem duplications (ITD) and Asp835 codon mutations of the FLT3 gene were reported as one of the most frequent genetic alterations in acute myeloid leukemia (AML). The recently described JAK2 V617F point mutation is supposed to be a causative acquired genetic alteration in chronic myeloproliferative disease (CMPD) that may transform into AML. Data about the frequency of JAK2 V617F point mutation among AML patients are rather limited. In the present study, 152 consecutive adults with newly diagnosed AML (57 males and 75 females) treated in our institute between January 2001 and December 2005 were enrolled. The median age of onset was 49 ± 14 (range 18-83) years. We analysed FLT3 ITD and Asp835 mutations by fluorescent PCR and PCR-RFLP methods; and JAK2 V617F by allele-specific PCR at the time of diagnosis. ITD was present in 28.5% (31/112) of the patients, Asp835 mutations were detected in 6.8% (8/119). Three patients (2.2%) carried both mutations. JAK2 V617F mutation was positive in 2.2% (3/132) of AML patients. 52% (66/131) of ITD-positive patients had monocyctic leukemia (M5 in the FAB classification system); while only 17% (17/101) ITD-negative patients had M5 leukemia (p=0.001). Regarding the prognostic significance of FLT3 mutations, only patients under 60 years of age receiving curative treatment (n=126; M/F =53/73) were considered. There was no difference in the complete remission rates (CR) between ITD positive and negative patients (76.9% vs. 70.1%), but the relapse rate (RR) was significantly increased in the ITD positive group (85.0% vs. 36.1%; p = 0.025). There was no ITD positive patient in the subgroup with favourable cytogenet-ic karyotype (n=17). The RR in the FAB-M5 group was not higher than in the whole cohort despite of the high frequency of ITD positivity. There was no difference in CR and RR between the Asp835 positive and negative groups. Only one out of the three AML patients with JAK2 V617 mutation had previous CMPD. None of the V617F positive patients carried the FLT3 mutation. Our results confirm earlier observations on Hungarian patients with AML that FLT3 ITD mutation is a negative prognostic factor. Our data raises the question whether in rare cases, the JAK2 V617F mutation may contribute not only to the development of CMPD, but also to AML.

### Table 1

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<tr>
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There was no significant correlation between graft failure and the number of CD34+ or CD38+ infused. Probability of graft failure at one year was 18.2 + 11.8% after FluBu and 4.6 + 6.6% after BuCy (difference not statistically significant). Acute GVHD grade ≥ 2 occurred only in 2 pt. Two of 5 pt with graft failure are alive 5 years after a 2nd graft, in CR and with chronic GVHD. Other 6 patients had chronic GVHD: limited in 3 pt and extensive in 8 pt. Overall survival (OS), disease free survival (DFS) and relapse rate are shown in the table. Overall survival was significantly better if the number of CD3+ cells was < 0.19 x 10^6/kg; the OS was 94.4 (+5.4%), 88.9 (+7.4) and 83 (+9)% at 1, 3 and 5 years, respectively, when CD3+ cells infused were > 0.19 x 10^6/kg. There was no significant difference in DFS between subgroups. Transplant related mortality at 100 days and at 1 year was 2.7 (+2.7) and 10.1 (+5.1)% respectively. In conclusion, in our experience ASCT with lymphocyte depleted grafts in AML is associated with a low risk of acute and chronic GVHD and seems to reduce transplant related mortality without an increase in relapse risk. These results confirm other studies of ASCT with lymphocyte depleted grafts in AML.

**1172**

**ALLOGENEIC STEM CELL TRANSPLANTATION WITH CD34+ SELECTED GRAFTS IN ACUTE MYELOID LEUKAEMIA: A LONG-TERM FOLLOW UP**


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GVHD is a major cause of morbidity and mortality after ASCT. In order to decrease transplant related toxicity, in 1996 we have launched a program of ASCT with CD34+ selected grafts in AML. Between August 1996 and July 2004, allogeneic stem cell transplantation with CD34+ positive selected PBSC has been performed in 57 patients with acute myeloid leukemia. In 6 pt the cytogenetics was of poor prognosis. In all cases the donors were HLA identical siblings. Median age: 36 years (5 - 55); sex: 15/22 (males/females). Status of disease at transplantation: 1st CR: 32; 2nd CR: 4; early relapse: 1. Myeloablative conditioning regimens used were: busulfan + cyclophosphamide + ATG (BuCy); 26: busul-fan + fludarabine (FluBu): 11; After July 2002 lymphocytes were added back to infuse a target number of 0.3 x 106 CD3- cells/kg receptor. GVHD prophylaxis was: cyclosporine-methotrexate: 20; cyclosporine only: 17. Median follow-up of surviving patients is 1813 days (range: 560 - 2942). Cells infused×10^6/kg (median (range)): CD34+: 5.1±10 (0.9 - 15.1); CD3: 0.19 (0 - 0.66). Thirty six evaluable patients engrafted. Five patients had late graft failure (median 54 days, range 113-1204).

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of their function are involved in leukaemogenesis and different mechanisms of transcription, mRNA expression and protein translation appear to be important in some AML subsets. Aims. We have investigated C/EBPα and PU.1 expression levels, and their reciprocal ratio, in different subsets of AML and correlated these data to morphology, FLT3 ITD mutations and cytogenetics. Methods. Bone marrow mononuclear cells (BMMCs) were isolated from 117 patients with AML at the time of diagnosis and from 13 normal bone marrows using Ficoll gradient. CD34+ cells were isolated from normal bone marrow by immunomagnetic separation. Granulocytes, monocytes and lymphocytes were isolated from buffy coat of normal donor. AML diagnosis was made according to WHO criteria. Cytogenetic data were available for 73 patients. C/EBPα and PU.1 levels were quantified by real time RT-PCR using 18S as reference gene. FLT3 ITD mutation was studied using current protocols. Results. Heterogeneous expression of PU.1 and C/EBPα was observed in different AML subsets. Higher levels of PU.1 and C/EBPα were observed in promyelocytic and myelomonoblastic leukaemias. We observed that PU.1/C/EBPα ratio reached statistical significance (p=0.0001 and p=0.05, respectively). Down-regulation of C/EBPα was observed in AMLs with t(8;21) and acute erythroid leukemias. We observed that PU.1/C/EBPα ratio was higher in monocyties and decreased progressively from peripheral granulocytes to CD34+ cells. When analysing the distribution of PU.1/C/EBPα ratio, AMLs with t(8;21) showed the highest ratio (median ratio=1.42), while acute erythroid leukemias had the lowest ratio (median ratio=0.43). This was particular, when comparing AMLs with other AMLs and to normal BMMC, the differences in PU.1/C/EBPα ratio reached statistical significance (p<0.0001 and p=0.05, respectively). Since down-regulation of C/EBPα and PU.1 has been described in cell lines expressing FLT3-ITD, we correlated their expression to FLT3 mutations. FLT3-ITD were present in 18 of 112 patients studied (16%) but no differences were observed in PU.1 and C/EBPα levels in mutated and unmaturated patients. Moreover, to verify the functional importance of these data, we studied the expression of two C/EBPα and PU.1 target genes, G-CSFR and M-CSFR respectively, in patients with high and low levels of these transcription factors. We found a direct correlation between levels of PU.1 and M-CSFR and between levels of C/EBPα and G-CSFR. Summary/Conclusions. C/EBPα and PU.1 expression and alteration of their reciprocal ratio may play a role in the pathogenesis of specific subsets of AMLs. Deregulated expression of these transcription factors may lead to an ineffective transcriptional control of hematopoiesis. 1174 HEART INFARCT AS THE MAJOR CAUSE OF EARLY DEATH OF HEMATOLOGICAL PATIENTS AS IDENTIFIED BY AUTOPSY A. Carvalhalis,1 F. Kaminski,2 A. Wawryszuk,2 A. Waszwczuk-Gajda,2 L. Koperski,2 A. Wasutynski2 Instituto Portugus de Oncologia, PORTO, Portugal; Medical University of Warsaw, WARSAW, Poland While majority of hematological patients die due to either their disease or to adverse reactions to their treatment, there is a paucity of studies that use autopsy to more precisely identify the actual causes of death in each case and to relate this to the clinical diagnosis. More precise knowledge of such causes in hematological malignancies and aplastic anemia would allow to properly focus research efforts and possibly to decrease mortality rates. In this study, the results of 154 autopsies of patients (the largest such series in the literature) with hematological diseases were reported and compared with clinical data. They concerned 13.6% of 1129 patients who died in this Department in the years 1996-2005. The most probable causes of death in particular hematological diseases, discordancies between clinical and autopsy diagnoses, and their relation to clinical characteristic were identified in the studied cohort. We were able to identify the cause of death up to 71% of cases. However, in 18% the cause of death was not explained by the clinical course and in 50% was sudden. Although various infections combined have been found to be responsible for the largest number of deaths (26.6%), the most common single cause was myocardial infarction (29 patients or 18.8%). Moreover, the myocardial infarction was found to be the most common cause of death in all hematological diseases (18-42, 45-62, 60-90 years). In majority of hematological malignancies (acute myeloblastic leukemia, acute lymphoblastic leukemia, non-Hodgkin’s lymphoma, Hodgkin’s lymphoma, multiple myeloma, chronic lymphocytic leukemia). Furthermore, this fatal myocardial infarction frequently occurred early after diagnosis and initiation of treatment, without preexisting coronary heart disease. The discordance between clinical and autopsy diagnosis of immediate cause of death was found in 55 patients (55.7%, 95% c.i. 28.2-42.8%) of which 59.0% of cases were considered class I discrepancy according to Goldman’s criteria. The myocardial infarction was found to be clinically undiagnosed in 69% of cases. In 41% it was class I discrepant diagnosis. These data suggest that hematological patients require special attention and probably preventive measures concerning myocardial infarction particularly during initiation of antineoplastic therapy. 1175 HUMAN CD34 POSITIVE RESISTANT MYELOID LEUKEMIA CELLS EXPRESS THE EMBRYONIC STEM CELL ANTIGENS: OCT-4 AND CD133 H. T. Hassan, X. Zhai, J. A. Goodacre Lancashire School Postgraduate Medicine, PRESTON, United Kingdom In 1942, Globus & Kuklenbeck proposed the presence of embryonic remnants in the sub-ventricular zone of human brain capable of giving rise to malignant tumours [Arch Pathology, 34, 674-734]. Recently small population of OCT-4 positive embryonic stem cells has been identified in adult murine bone marrow. Also, human CD3+ positive cord blood stem cells co-express OCT-4 and other embryonic genes. The aim of the present study was to investigate the presence of embryonic stem cells markers in human AML CD34 positive cells.

We examined the presence of the two isoforms of human stem cell CD133 antigen and the embryonic OCT-4 antigen in KG1a human CD34 positive resistant AML cells. Both immunofluorescence and immunocytochemical stainings of AML CD34 positive resistant cells with anti-CD133 epitope-1 (clone AC133, Miltenyi Biotec Ltd) and anti-CD133 epitope-2 (clone 293C3, Miltenyi Biotec Ltd) and anti-OCT-4 (clone sc-5279, Santa Cruz Biotechnology Inc.) revealed the presence of OCT-4 and CD133 epitope-2 antigens but not CD133 epitope-1 antigen. More than 90% of KG1a AML cells were OCT-4 positive in three experiments using both negative and positive controls. OCT-4 positive cells have significantly larger size than negative cells. The presence of CD133 epitope-2 and not epitope-1 in these AML CD34 positive cells is in line with the CD133 epitope expression in normal endothelial and haematopoietic stem cells. The expression of OCT-4 embryonic antigen in both normal bone marrow and leukaemia cells provide new support for the 60-year old hypothesis of ‘embryonic remnants’ in adult life being target for and incapable of malignant transformation. Further studies are warranted to evaluate the presence of embryonic stem cell antigens in AML blast cells from patients and their functional relevance to resistance to chemother-apy and any prognostic value. 1176 LEUKEMIC INFILTRATION OF THE RETINA AT ONSET OF PHILADELPHIA POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA REVEALED BY STRATUS OPTICAL COHERENCE TOMOGRAPHY (OCT) A. Candoni, E. Simeone, S. Buttignol, S. Lovato, R. Fanin University Hospital, UDINE, Italy A 55-year-old man was admitted with pancytopenia and a loss of vision with a central visual field defect in his left eye. Bone marrow evaluation revealed acute lymphoblastic leukaemia, B-lineage phenotype, cytogenetic and molecular genetic analysis showed t(9;22) and a P210 positivity (BCR-ABL, a2b2). The cerebrospinal fluid was positive for leukaemic cells (100 blast cells/μL). Ophthalmic examination was performed. Fundoscopy showed a lifting and detachment of neuroretinal epithelium in the left eye, which was confirmed by ultrasound examination (vitreous cavity was normal). Stratus Optical Coherence Tomography (OCT) showed a retinal detachment with a choroidal infiltrate in the left eye (Figure 1). Brain and orbital magnetic resonance imaging were normal. The patient underwent induction chemotherapy with daunorubicin and vincristine and intrathecal chemotherapy with
methyltrexate, cytarabine and desamethasone. Funduscopic and OCT, after one course of systemic chemotherapy and two courses of intrathecal chemotherapy, showed complete regression of the retinal infiltration with full recovery of visual function (Figure 2). OCT is a non-invasive way to study the retina that uses reflection of light off the retinal layers to create a high resolution colour tomographic image of retinal structures with an axial resolution of 10 microns or less. In leukemic patients with a suspicion of posterior ocular segment involvement this technique can be considered as a new and non-invasive diagnostic procedure to see beneath the surface of the retina, permitting detection and follow up of leukemic infiltrates.

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TREATMENT RESULTS OF ACUTE LYMPHOBLASTIC LEUKEMIA IN CHILDREN ACCORDING TO ALL-BFM 95 PROTOCOL

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Aims. To evaluate feasibility of treatment of acute lymphoblastic leukemia (ALL) according to the BFM 95 protocol at a single centre in Bulgaria; to assess the 10-years disease-free survival (DFS) in children from all the risk groups; to find factors with prognostic impact on survival from ALL. Methods. We studied a cohort of 104 children (59 boys and 45 girls) with ALL, treated at a single centre from January 1996 to January 2006. The median age of the study group was 6.7 years (from 2 months to 18 years). The chemotherapy and treatment stratification were identical to the ALL-BFM 95 protocol. Patients were stratified into 3 risk groups, based on age, initial white blood cells' count, immunophenotyping, cytogenetics and response to initial treatment: standard-risk (SR), intermediate-risk (IR) and high-risk (HR) groups. Response to initial treatment was assessed by the steroid response on day 8 and hematological remission on day 33 after beginning of chemotherapy. Survival curves were calculated according to the Kaplan-Meier method and statistical significance of differences between curves was determined by the log-rank Mantel-Cox test. Logistic regression was carried out for assessing factors with prognostic impact on survival. Results. The patients from the study group were stratified in SR: 31 (29.8%), IR: 52 (50%) and HR: 21 (20.2%) patients. CNS involvement was proven in 2 (1.9%) patients, mediastinal mass - in 14 (15.5%) patients, renal infiltration - in 13 (12.5%) patients. Poor steroid responders were 20 (19.2%) patients and remission was not achieved on day 33 in 6 (5.8%) patients. The 10-years DFS probability was 81.4 ± 1.3% for the SR group, 72.6 ± 0.7% for the IR group and 58.6 ± 1.3% for the HR group (p = 0.027). Independent prognostic factors for DFS, when conducting risk-adapted chemotherapy, proved to be radiologically-proven mediastinal mass (p = 0.008; RR: 5.7, 95% CI: 3.2-416.9) and timely remission induction (p = 0.002; RR: 24.6, 95% CI: 2.1-283.9). The majority of relapses occurred within 3 years from diagnosis and most involved the bone marrow. Conclusions. The DFS of the studied group is compatible with the reported from the official BFM study group. Our results on the basis of risk-adapted treatment suggest lack of correlation between survival and the prognostic factors considered previously as significant.

1178
COMPARISON OF HLA CLASS I (A, B, C) AND CLASS II (DRB) POLYMORPHISMS IN IRANIAN PATIENTS WITH ACUTE LYMPHOBlastic LEUKEMIA (ALL) AND CONTROL GROUP

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Backgrounds. HLA gene polymorphisms have been extensively studied in various immune-mediated as well as malignant diseases such as leukemia. Since the first study on mouse leukemia by Lilly et al in 1964, the role of MHC molecules as genetic factors affecting the susceptibility or protection against leukemia have been proposed. Aims. The aim of this study was to compare the HLA class I (A,B,C) antigens and class II (DRB) alleles frequency between a group of 141 Iranian patients with Acute Lymphoblastic Leukemia (ALL) and two distinct control group of 100 and 180 healthy individuals for HLA class I and II analysis, respectively. Methods. From all of the patients and control subjects blood samples were collected after giving informed consents. HLA class I antigens were determined using serology method while DNA extraction and HLA-DRB typing were performed using PCR-SSP analysis. Results. Significant increased frequencies of HLA-A*130: 12.4% vs. 1% (p=0.002), OR=0.074, 95% confidence interval (CI): 0.009-0.58 and HLA-Cw*07: 34.8% vs. 20% (p=0.03, OR=2.15, 95%CI: 1.13-4.08) were observed in patients with ALL when compared with control group. Patients showed significant lower frequency of some antigens including HLA-B*05 (p<0.0001), B*12 (p=0.005), B*14 (p=0.005), B*41 (p=0.005), B*61 (p<0.0001), B*65 (p=0.0000), B*52 (p=0.0000) vs. healthy control group. No significant differences were found between patients and control group when compared for HLA-DRB allele frequencies. Conclusion. This study suggested the role of some HLA antigens including HLA-A*130 and HLA-Cw*07 as predisposing factors in susceptibility to ALL. While through the patients with lower frequencies in ALL patients, HLA-B*05, B*61 and Cw*07 showed a stronger and more significant differences. Future studies are needed to confirm these associations in larger samples and investigate the role of specific subtypes using molecular techniques.
1180 ANOREXIA-CACHEXIA RELATED HORMONES AT DIAGNOSIS AND DURING CHEMOTHERAPY IN CHILDREN WITH ACUTE LYMPHOBlastic LEUKAEMIA

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Backgrounds. Anorexia and cachexia are common manifestations in children with acute lymphoblastic leukaemia (ALL) at diagnosis. Possible mediators of the anorexia-cachexia syndrome are hormones, cytokines, and adipokines from peripheral tissues, and neurotransmitters, neuropeptides, cytokines, and other hormones in the hypothalamus. Peptide YY (PYY) and ghrelin are gastrointestinal tract-derived hormones involved in the short- and long-term regulation of food intake and energy balance. PYY, synthesized mainly by endocrine cells of the terminal ileum and colon, is released into the systemic circulation in response to a meal and participates in signalling the end of the meal at the hypothalamic phase of satiety action possibly through an Y2 receptor-mediated mechanism. Ghrelin, secreted predominantly from X/A-like endocrine cells of the oxyntic glands of the stomach, is primarily secreted in the fasting state, with plasma concentrations falling within one hour of a meal. Its role in food intake and energy balance is opposite to that of PYY, as it exerts orexigenic effects through activation of the hypothalamic neuropeptide Y Y1 (NPY-Y1) pathway. Aim: We evaluated the secretion of PYY and ghrelin at diagnosis and during chemotherapy in children with ALL. Methods. Ten patients aged 2-7 years were included in this prospective study. All patients were treated following the same protocol (HOPDA97). A physical examination was performed and blood chemistries were evaluated by standard techniques. Preprandial PYY and active ghrelin levels were determined by specific radioimmunoassays (Linco Research, Inc., USA). Measurements were performed at diagnosis prior to chemotherapy and at several time points prior to each next cycle of chemotherapy for up to 18 months (6-10 measurements per patient). Results. Baseline PYY levels were 213.2±85.3 pg/mL, increased significantly to 283.9±72.9 pg/mL after the induction and consolidation phase of chemotherapy, and returned progressively to pre-treatment levels at the 6th cycle of the maintenance phase. Baseline active ghrelin concentrations at diagnosis were low (52.6±8.6 pg/mL), fluctuated throughout the study period and stabilized at significantly higher levels (57.4±31.6 pg/mL) after the 8th cycle of maintenance chemotherapy. Conclusion. These data suggest that in children with ALL and anorexia-cachexia the levels of PYY decrease with time, as the leukemic burden is eliminated. In contrast, active ghrelin levels are relatively low at diagnosis, remain low during the early cycles of chemotherapy, but normalize with the elimination of the leukemic burden, paralleling the body weight gain trajectory.

References

1181 SCREENING FOR EVI1 ECTOPTIC EXPRESSION IN T-ALL PATIENTS

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Backgrounds. Balanced chromosomal rearrangements involving chromosome band 3q26 due to translocations with various partner chromosomes are a recurrent finding in myeloid malignancies. These translocations either contribute to the ectopic expression of, or to the formation of fusion genes involving the EVI1 proto-oncogene. EVI1 transcriptional activation has been reported in up to 10% of acute myeloid leukemias (AML), and is an independent indicator of adverse prognosis. While EVI1 expression is a well documented oncogenic event in myeloid malignancies, EVI1 was presumed not to be involved in lymphoproliferative disorders. However, in an extensive molecular characterization of unselected 3q26 rearrangements, we recently reported the sporadic occurrence of balanced 3q26 aberrations in lymphoid neoplasms. In a t(3;9)(q26;p23) identified in a T-cell Non Hodgkin’s lymphoma, FISH confirmed a genomic EVI1 rearrangement. Since these observations suggested a possible involvement of EVI1 in T-cell malignancies, we initiated additional analyses designed to define the presence and frequency of ectopic EVI1 expression in T-ALL. Aim of the study. The aim of this study was to investigate the possible ectopic EVI1 expression in T-ALL. Methods. We performed real-time quantitative PCR using validated EVI1 primer pairs (1), dedicated to the sensitive detection of ectopic EVI1 expression, on a multi-centre collected series of 87 T-ALL patients and 5 T-ALL cell lines. Results. EVI1 overexpression was demonstrated to be absent in the 87 patient samples and the 5 tested T-ALL cell lines. Conclusion: Although EVI1 overexpression is a poor prognostic marker in AML, it seems not to be involved in the pathogenesis of T-ALL.

References

1182 ECONOMIC AND QUALITY OF LIFE BURDEN OF HIGH-RISK ACUTE LYMPHOBLASTIC LEUKEMIA

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Backgrounds. Patients with high-risk acute lymphoblastic leukemia (ALL), including Philadelphia chromosome positive (Ph+) ALL, typically have extremely poor prognosis, experience poor quality of life (QoL) and incur high economic cost. Aims. This study examined the economic and QoL outcomes for high-risk ALL patients including Ph+ ALL. Methods. A systematic search of the English-language literature published between 1990 and 2005 was conducted. Additional searches were conducted from the retrieved article bibliographies and appropriate conference proceedings (2000-2005). Articles selected for inclusion were prospective or retrospective studies specifically designed to examine burden of illness, direct medical costs, cost drivers, or QoL outcomes of ALL and treatments. Results. Of 798 abstracts screened, 106 met selection criteria and were reviewed in detail. Forty-nine and 47 studies focused on the economic and QoL aspects of ALL, respectively. Patients with high-risk ALL are usually defined by cytogenetic alterations (e.g., t(9;22)(q34;q11), t(4;11)(q21;q23)), age, increased white blood cell count, and slow response to therapy. The average annual direct medical cost per high-risk ALL patient ranged from $100,000 to $150,000 as compared to $40,000 to $74,000 for a standard-risk ALL patient. Hospitalization was the major cost component comprising 50%-80% of total direct costs. Major hospital cost drivers included infections, chemotherapy, growth factors, transfusions, and transplantation. These drivers resulted in more frequent hospitalizations and longer ICU lengths of stay for high risk patients. High-risk ALL patients typically had psychological and physical complaints, especially in domains of emotion, cognition, and pain. Furthermore, high-risk patients were more likely to have poorer QoL than standard-risk patients due to higher relapse rates and increased need for transplantation. Conclusions. ALL exacts a substantial economic and QoL burden on patients, their loved ones and society in general. This burden appears particularly heavy for high-risk patients, such as patients with Ph+ ALL, one of the worst prognosis in ALL. Imatinib either as a single agent or as part of combination regimens has been reported to extend disease-free-survival and improved quality of life among patients with Ph+ ALL in clinical studies (Pui et al. NEJM 2006). Further research is undertaken to evaluate the economic and QoL benefits of imatinib as compared to the current therapies in the treatment of Ph+ ALL.

1183 TREATMENT OF RELAPSED PHILADELPHIA-POSITIVE ACUTE LYMPHOBLASTIC LEUKAEMIA AFTER MYELOABLATIVE STEM CELL TRANSPLANTATION WITH IMATINIB

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Backgrounds. Philadelphia chromosome-positive acute lymphoblastic leukemia have a markedly poor prognosis when treated with conventional chemotherapy alone. Even with intensive treatment such as allogeneic transplant, a large proportion of patients relapse. Aims and Methods. We described here two patients with Ph+ ALL who relapsed after HLA-identical sibling donor stem cell transplantation and were treated...
with shortened course of induction chemotherapy and imatinib mesylate (400 mg/day). Results. One of these (male age 36) received imatinib for MRD positivity detected in PCR after SCT. Bcr-abl transcript became undetectable after 1.5 month of imatinib treatment. STI and immunosuppressive therapy was discontinued at day +120. These response was not sustained and the patient relapsed 9 month after alloSCT because of chronic GVHD. He was resistant to conventional therapy with imatinib combined with additional mild chemotherapy and achieved complete donor chimerism with PCR negativity. Because of extensive chronic GVHD (skin, oral sicca, ocular sicca, bronchiolitis obliterans without thrombocytopenia) systemic steroids therapy was introduced and imatinib (400mg/day) was simultaneously continued. At present, the patient has a 19-month post-transplantation follow-up and is in stable molecular remission as evaluated by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) for the BCR/ABL fusion gene transcript. GVHD has partially resolved and patient was able to reduce immunosuppression. In the notably during imatinib treatment we have observed unusual clinical improvement of bronchiolitis obliterans (BO) process confirmed by computed tomography and pulmonary function testing. Another patient relapsed 6 month after alloSCT and obtained complete hematological remission with 100% donor chimerism and PCR negativity after mild chemotherapy followed by imatinib. Imatinib was well tolerated and did not induce GVHD. At 15 months follow-up patient is still in complete hematological and molecular remission. DLI was planned. Conclusions. Imatinib combined with low dose chemotherapy is a promising therapy option in Ph+ALL patients relapsed after alloSCT not eligible for intensive treatment, achieving remission prior to DLI and maintain remission during immunosuppressiv treatment. Further studies to elucidate a role of imatinib (VEGF gene transfer and platelet-derived growth factor receptor inhibitor) in transplant BO are needed.

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BURKITT-LIKE LYMPHOMA: A NEW TREATMENT PROTOCOL BL-L-M-04
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Backgrounds. Burkitt-like lymphoma (BL) is a clinical and morphological variant of Burkitt lymphoma. It is the most aggressive B-cell lymphoid neoplasm, which proliferative activity approximates 100%. At the same time BL is one of the most chemosensitive lymphoma. Intensive chemotherapy allows achieving remissions in 70-90% cases and increasing a common 5-year survival up to 60-75% (according to the stage of disease). Aim: to evaluate an efficacy and toxicity of protocol BL-L-M-04. Methods. Seventeen patients (12 males and 5 females, mean age 25 years) were eligible for inclusion in the study if they had a diagnosis of Burkitt-like Lymphoma (BL). All the patients participated in the study performed in Russian Hematological Research Center between January 2001 and May 2005. Fourteen patients were eligible for the initial chemotherapy. Three patients from other clinics had a diagnosis of Burkitt-like Lymphoma and were treated with CHOP (cyclophosphamide, vincristine, adriamycin and prednisolone), but in Russian Hematological Research Center were used additional diagnostic methods (fluorescent in situ hybridisation, flow cytometry, immunohistochemistry) and BL staging criteria developed by S. B. Murphy was used to stage the patients. The stage I, II, III, IV was diagnosed in 2, 1, 6, 8 patients respectively. Bone marrow was involved in 5 patients, neutrophilemia - in 5 patients. B-symptoms (night sweats, fever, weight loss) were in 13 patients. Serum lactate dehydrogenase level was increased in 15 patients. The most frequent complication of treatment was infection (Grade 3 or 4 non-hematologic toxicities were; mucositis (67%), infection (39%), peripheral neuropathy like Guillain-Barre syndrome has been reported. Among these poor treatment-related complications (2 patients) or progressive disease (1 patient). Median overall survival (OS) was not reached yet, and 1-year OS rate was 64% (95% CI, 50-76%). All patients treated with BL-L-M-04 had hematologic toxicities of grade 3 or 4 neutropenia/thrombocytopenia. Grade 3 or 4 non-hematologic toxicities were: mucositis (67%), infection (58%), hepatic toxicity (42%), peripheral neuropathy (25%), and azotemia (25%). Summary/Conclusions. Russian patients with Burkitt’s lymphoma were treated following the protocol proposed by the Cancer and Leukemia Group B (CALGB) 9251, grade 4 mucositis was reported in every patient. Thus, we had modified CALGB 9251 protocol and named BNHL. Aims. This study was aimed to show the clinical manifestations and to evaluate efficacy and toxicity of BNHL in patients with Burkitt’s lymphoma in a Korean single center. Methods. Between January 1998 and July 2005, 25 patients were diagnosed as Burkitt’s lymphoma at Asian Medical Center were available to evaluate the clinical features. Among 25 patients, 12 patients treated with BNHL were available. In BNHL protocol, we reduced the dose of methotrexate (1,500~1,000 mg/m^2/day on day 1 of cycles 1, 3, and 5) and etoposide (30~50 mg/m^2 on days 4 and 5 of cycles 2, 4, and 6). Results. Median age was 50.4 years (range: 17-77) and 15 patients were male. Among 25 Burkitt’s lymphoma, 20 patients had extranodal involvement, and 9 patients had 2 or more extranodal involvements. Extranodal sites were bone marrow, GI tract, genitourinary organ, bone, and lung, in decreasing order. Among total patients, 16 were in stage III or IV, and 9 were in stage I or II. Twelve patients had B symptoms, and 16 patients had high or intermediate score of age adjusted international prognostic index (IPI). For 12 patients treated with BNHL, median follow-up duration was 13 months (range: 3-20 months). Among patients treated with BNHL, 9 patients achieved CR, and 3 patients achieved PR. The event free survival (EFS) rate at 1 year was 54% (95% CI, 39-69%) and median EFS was not reached. Among 9 patients who achieved CR, 2 patients were relapsed. Three patients died as a result of treatment-related complications (2 patients) or progressive disease (1 patient). Median overall survival (OS) was not reached yet, and 1-year OS rate was 64% (95% CI, 50-76%). All patients treated with BNHL had hematologic toxicities of grade 3 or 4 neutropenia/thrombocytopenia. Grade 3 or 4 non-hematologic toxicities were: mucositis (67%), infection (58%), hepatic toxicity (42%), peripheral neuropathy (25%), and azotemia (25%). Summary/Conclusions. Korean patients with Burkitt’s lymphoma had worse age-adjusted IPI score than those in western countries. Treatment-related complications were not frequently reported because these poor risk factors, BNHL modified from CALGB 9251 showed satisfactory efficacy and acceptable toxicities in Korean Burkitt’s lymphoma.

1186
ACUTE PERIPHERAL NEUROPATHY FOLLOWING HYPERCVAD REGIME FOR MANTLE-CELL LYMPHOMA
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Backgrounds. HyperCVAD is an effective regimen for the mantle cell lymphoma (MCL) with potential cerebellar toxicity, but non acute peripheral neuropathy like Guillain-Barre syndrome has been reported. We report three patients with MCL that presented a severe acute peripheral neuropathy probably secondary to the hyperCVAD/MITX-AraC reg.

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**PHASE II CLINICAL EXPERIENCE WITH BORTEZOMB IN PATIENTS WITH INDOLENT NON-HODGKIN'S LYMPHOMA AND MANTLE CELL LYMPHOMA**

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**Backgrounds.** Bortezomib is a novel small molecule, which is a potent selective inhibitor of the 26S proteasome. Bortezomib has also shown activity in vitro against a variety of lymphoma cell lines including mantle-cell lymphoma (MCL)-derived and diffuse large-cell lymphoma-derived cell lines. Aims. The preclinical results and clinical observations provided the rationale for this Phase II trial of Bortezomib for the treatment of patients with relapsed or refractory B-cell non Hodgkin lymphoma. Methods. To date, we have treated 11 previously treated patients (pts.), (8 males and 3 females with a median age of 60 years, median duration of prior therapies 4) with relapsed or refractory indolent lymphomas including: 2 pts. with small lymphocytic lymphoma; 2 pts. with follicular lymphoma; 7 pts. with MCL. Patients were treated at a dose of 1.5 mg/m² twice weekly for two consecutive weeks with a one-week rest period. Results. No grade III or IV toxicities were observed, save one patient that developed a grade 3 sensory and motor neuropathy. Re-staging studies were routinely performed after two complete cycles of therapy. All pts. with small lymphocytic lymphoma and follicular experienced PD. Among the seven assessable pts. with MCL there was two pts. CR, two pts. PR, one patient stable and one in two pts. with PD. In responders pts., the median time to progression was not reached with a median follow-up of 9.1 months. Conclusions. These data continue to support the biological activity of Bortezomib in pts. with select sub-types of indolent non-Hodgkin's Lymphoma.

**1188**

**LIPOSOMAL DOXORUBICIN IN THE TREATMENT OF Lymphoma PATIENTS**

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**Backgrounds.** Myocet (liposomal doxorubicin) has an improved pharmacokinetic profile with less myelosuppression and GI toxicity and has a reduced risk of cardiotoxicity at dose level equivalent to standard formulations of doxorubicin. Methods. From June 2003 we replaced the conventional doxorubicin with liposomal doxorubicin (Myocet 50 mg/m² in COMP and 25 mg/m² in MVBD) for the treatment of 25 patients (pts.). The pts. were selected pts: elderly pts, pts with impaired cardiac function, pts previously treated with less myelosuppression and GI toxicity and at the end of therapy. Two pts with NHL were treated with R-COMP and 5 Hodgkin's lymphoma with MVBD. Results. The median age was 68 years (range 54-76). Three pts were stage I, 7 stage II, 6 stage III and 9 stage IV. According to IP1 score, for NHL only, 7 pts were low risk, 6 low-intermediate, 6 intermediate-high and 1 high risk. Seven were pretreated with doxorubicin (490 mg median cumulative dose), 7 pts showed impaired cardiac function (4 ischemic, 7 hypertensive and 2 hypokinetic). The median left ventricular ejection fraction (LVEF) at diagnosis was 59% (range 45%-70%). We performed cardiac evaluation at diagnosis, after three cycles and at the end of therapy. All pts but one had no change in LVEF; one patient (4%) presented a myocardial disfunction resolved with medical therapy. The average dose of liposomal doxorubicin for patients who concluded therapy was 465 mg (range 80-600 mg). At the moment 21 out 25 patients are evaluable for response: 15 pts obtained a complete remission (71%) three a partial remission with an overall response of 86%, one patient stopped therapy due to myocardial disfunction and two patients died one for a stroke and the other for gastrointestinal bleeding. After 130 cycles we have observed one toxic event and two concomitant complications. No significant hematological toxicity was recorded. Three pts died of disease and after a median observation period of 12 months (range 1-32) the overall survival was 80%. Conclusions. We conclude that liposomal doxorubicin allows to treat patients with concomitant diseases which could limit the use of conventional anthracycline. Myocet is feasible and effective in a subset of patients with very negative characteristics at diagnosis. It reduces cardiotoxicity risk without reducing chemotherapeutic efficacy.
enced grade 4 neutropenia and 6 patients (40.0%) experienced grade 4 thrombocytopenia. Autologous stem cell collection was attempted in the 7 patients and was successful in all cases. The median number of CD34-positive cells collected was 5.2 (range, 2.8-11.6)×10^6/kg. Of 13 patients < 66 years, 4 patients (30.8%) proceeded to stem cell transplantation. Conclusions. GEPD chemotherapy in patients with primary progres- sive or relapsed NHL is effective as salvage therapy and does not interfere with the ability to harvest autologous stem cells for subsequent transplantation. A final analysis is planned after total 40 patients are enrolled.

**1190**

**R-FND VS R-CHOP TREATMENT AS FIRST LINE THERAPY FOR FOLLICULAR LYMPHOMAS: A SINGLE INSTITUTIONAL EXPERIENCE**

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*Background.* High response rates in follicular lymphoma (FL) with the FMD protocol have been previously reported. The monoclonal anti-CD20 antibody Rituximab has been shown to induce a high response rate in FL patients and to improve outcome when associated with clas- sic regimens (CVP or CHOP). *Aims.* We have evaluated the impact of R- FMD as compared to R-CHOP as a first line therapy in patients with fol- licular lymphomas, in terms of: complete response (CR), overall survival (OS), toxicity and the efficacy of PCR molecular analysis in predicting clinical and molecular remission. *Methods.* Between September 2002 and April 2006, 13 FL patients were enrolled in the study. Fourteen patients (M/F: 8/6, median age 54 years) received R-FMD treatment in stage I- II, FLIPI score: intermediate grade 3 pts, high grade 11 pts. R-FMD regi- men was administered every 28 days for six cycles: Fludarabine 30 mg/m² e.v. days 1-3, Mitoxantrone 10 mg/m² day 1, Dexamethasone 20 mg days 1-3 and Rituximab 575 mg/m² day 1. PCR molecular analysis was performed in 12 patients at diagnosis, showing in 10 (84%) of them bcl-2 rearrangement. Fourteen patients (M/F: 7/7, median age 56 years) received R-CHOP treatment in stage II-IV, FLIPI score: intermediate grade 4 pts, high grade 10 pts. R-CHOP regimen was delivered every 21 days for six cycles, preceded on day 1 by Rituximab 575 mg/m². PCR molecu- lar analysis was performed in 10 patients at diagnosis showing in 9 (90%) of them bcl-2 rearrangement. *Results.* Arm R-FMD: An overall response (13 (93%) CR and 1 (7%) partial response) was achieved in all patients; the pts in CR achieved CR after Zevalin. Actually, after a median follow up of 28 months, 14 pts resulted in CR. At the end of treatment, bcl-2 appeared to be negative in 8/10 pts (75%). The toxic- ity was mild with grade 3-4 neutropenia in 2 pts (14%), CMV infection was observed in one pt. Arm R-CHOP: Thirteen pts (93%) achieved CR and 1 resulted non responder. Out of all 13 pts in RC: 1 died in CR for infection, 1 relapsed after 23 months. After a median follow up of 25 months, 12 (86%) pts are alive, 11 (78%) of which are in continue CR. Grade 3-4 neutropenia was observed in 4 (28%) pts. At the end of treat- ment bcl-2 appears to be negative in 6/9 pts (66%). *Conclusion.* Our data demonstrate that both frontline R-FND and R-CHOP treatments produce high rate of response in terms of CR, OS and molecular remission and low toxicity. A more prolonged follow-up will be needed to determine the long-term efficacy of these combinations.

**1191**

**T(14;18), F53 AND RAS GENE MUTATIONS IN PATIENTS WITH RESIDUAL LYMPHOMA CELLS**

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PCR analysis of rearranged antigen receptor genes reveals sensitivity of 10^-5^ and has been demonstrated as valuable tool for detection of min- ormal residual disease (MRD) in lymphoma malignancies. However, the finding that patients with evidence of MRD sometimes remain in long- lasting remission directs further investigations toward biology of resid- ual disease. The aim was to correlate MRD results with the incidence of relapse and DFI, respectively. Furthermore, the presence of F53 and RAS gene mutations and t(14;18) was analysed in patients with residual malignt cells. The study included 40 B-NHL patients diagnosed and managed in MMA. 13/40 patients had high- (HG) and 27/40 had low- grade (LG) lymphoma. Seven patients achieved partial (PR) and 33 patients achieved complete clinical remission (CCR) after chemotherapy. Peripheral blood samples were analysed for MRD at up to ten fol- low-up points. All analysis included PCR amplification followed by appropriate electrophoresis. MRD was found in 13/33 patients (12 LG and 1 HG) who achieved CCR. The incidence of relapse was signifi- cantly higher in MRD+ vs MRD- B-NHL patients (Fisher's exact test, p=0.0085). In the LG group significant difference was not found. The only MRD+ HG patient relapsed. Significant difference in DFI between MRD+ and MRD- patients was not observed. Concerning MRD+ patients in CCR and patients who achieved PR, t(14;18) was found in six patients (4 relapsed). In the same group F53, K- and N-RAS mutations were not found. H-RAS mutations were found in six patients - 3 relapsed and 3 remains in CCR. Our results demonstrated positive correlation between MRD - positivity and incidence of relapse in B-NHL patients, but didn’t indicate significance of F53 and RAS mutations for evaluation of residual clone malignancy.

**1192**

**DOSE-ADJUSTED EPOCH-RITUXIMAB IS HIGHLY EFFECTIVE AND TOLERABLE IN UNTREATED POOR-PROGNOSIS DIFFUSE LARGE B-CELL LYMPHOMA: RESULTS OF A PROSPECTIVE STUDY**

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Less than 50% of patients with poor-prognosis diffuse large B-cell lymphoma (DLBCL), defined as score 2 and 3 (intermediate-high or high) according to the age-adjusted International Prognostic Index (aaIPI), remain disease-free for lengthy periods. The optimal therapeutic strategy for these patients is still much debated. This study was to evaluate the efficacy and toxicity of Rituximab and dose-adjusted EPOCH (DA- EPOCH-R) regimen for untreated poor-prognosis DLBCL. DA-EPOCH- R regimen (doxorubicin, vincristine, etoposide over 96 hours’ infusion with bolus cyclophosphamide, prednisone, and rituximab) was adminis- tered to 31 consecutive patients with previously untreated poor-prog- nosis DLBCL. At the end of DA-EPOCH-R, consolidation radiotherapy (36 Gy) was given to areas of previous bulky disease (≥10 cm). The last 8 patients received an intensive central nervous system (CNS) prophylaxis consisting of 3 courses (after cycles 2, 4 and 6 of DA-EPOCH-R) of orally dexamethasone (40 mg on days 1-4) and intravenous high-dose methotrexate 3 g/m² (1.5 g/m² in patients > 60 years of age), 125-I infu- sion, day 1 (with folic acid rescue). Median cycles of EPOCH-R regimen administered were 6 (ranged from 2 to 8 cycles). Younger patients (aged < 60 years) required higher dose rates than older patients (>60 years) to achieve the targeted absolute neutrophil count (ANC) nadir. Two-hun- dred and six cycles of chemotherapy were administered to 81 patients. Of the 31 patients enrolled in the study, 28 were evaluable for response. Overall, 92% of patients had an objective response; 78% (22/28) achieved a complete response (CR) and 14% (4/28) had a partial response (PR) at the end of therapy. At a median follow-up of 23 months (range 9-45), the event-free survival (EFS) and overall survival (OS) were 71% and 85% respectively. Two CR patients (both with an aaIPI score of 3) relapsed. Only aaIPI score of 3 demonstrated to have an adverse prog- nostic value. Toxicity at least grade 3 according to the WHO toxicity cri- teria (incidence by cycle) were: neutropenia (55%), thrombocytopenia (25%), anemia (15%), and oral mucositis (6%). Severe infections occurred in 25% of the cycles in patients older than 60 years of age com- pared with 10% of cycles in patients younger than 60 years of age. Four patients with previous cardiac disease and 3 patients with HCV antibody showed no severe cardiac nor hepatic toxicity during chemotherapy. There were 2 toxicity-related deaths (treatment-related mortality, 6%): one patient had an early toxic death due to neutropenic sepsis at week 16, and the other patient had a late toxic death due to secondary acute myeloid leukemia (FAB M4) that occurred at 8 months after the end of DA-EPOCH-R. The data from our institution are promising and add to the available evidence supporting the efficacy and safety of DA-EPOCH- R therapy for the treatment of poor-prognosis DLBCL, especially in patients with an aaIPI score of 2.

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**CHOP VS. RITUXIMAB-CHOP IN DIFFUSE LARGE B-CELL LYMPHOMA: A RETROSPECTIVE COMPARISON OF RESPONSE RATES AND OUTCOME**

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*Background.* Rituximab is an anti-CD20 monoclonal antibody that haematologica/the hematology journal | 2006; 91(s1) | 431
induces cytotoxicity via antibody-dependent cell-mediated and complement-dependent mechanisms, as well as via direct apoptotic signaling. Combination of rituximab with chemotherapy has an additive or synergistic effect and has been reported to increase response rates and prolong remission and survival in patients with diffuse large B-cell lymphoma (DLBCL).

**Aim.** To evaluate and compare retrospectively the response rates and outcome of a large number of patients with DLBCL according to the kind of treatment administered, CHOP-like regimens delivered alone (group A) or group B, that additionally received rituximab 375 mg/m² IV on day 1 of each chemotherapy cycle. Patients in both groups underwent a median number of 6 (1-8) cycles. Radiotherapy was additionally administered in 24 (21.2%) patients of group A and in 38 (30.8%) patients of group B (p > 0.05).

**Patients’ characteristics (gender, age, nodal or extranodal primary site of origin, stage, IPI, presence of B symptoms, extranodal involvement other than primary, bulky disease and bone marrow infiltration), as well as response rates, were compared between the two groups using χ² tests. Disease-free survival (DFS), overall survival (OS) and failure-free survival (FFS) were estimated according to the Kaplan-Meier method. Differences in survival rates were assessed using the log-rank test. Patients were well-balanced regarding their characteristics (p > 0.05). Median follow-up time for groups A and B was 62 (1-99) and 29 (1-62) months respectively (p > 0.001). On an intention-to-treat basis, complete response rates were similar between groups A and B (88.5% vs. 89% respectively, p > 0.05). Actuarial 3-year DFS rate was significantly higher in patients of group A compared to group B respectively (p = 0.046). Actuarial 3-year OS and FFS rates were not significantly different between groups A and B (77.7% vs. 70% and 62.5% vs. 69.7% respectively, p > 0.05). Conclusion. According to our results, the addition of rituximab to chemotherapy yields a higher DFS rate than chemotherapy alone with DLBCL. Nevertheless, our study failed to confirm the superiority of the rituximab-chemotherapy combination in terms of OS and FFS rates, probably due to the significantly shorter follow-up of this group of patients.

**1194 MYELOABLATIVE CHEMOTHERAPY AND AUTOLOGOUS STEM CELL TRANSPLANTATION IN POOR PROGNOSIS PATIENTS WITH ADVANCED DIFFUSE LARGE B-CELL AND FOLLICULAR LYMPHOMA EFFECTIVE THERAPY IN FIRST REMISSION**

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**Backgrounds.** Conventional chemotherapy in advanced, poor-prognosis diffuse large B-cell (DLBCL) and follicular lymphoma (FL) is still unsatisfactory in a number of patients ultimately dying of disease. The addition of rituximab to initial combination chemotherapy (e.g. R-CHOP and R-CVP) increases the number of complete remissions (CR), prolongs duration of response and survival, but the overall results in high-risk prognostic groups are suboptimal. **Aims.** Number of studies confirms that high-dose chemotherapy and autologous stem cell transplantation (AT) in younger pts. with accumulation of several adverse prognostic factors improves their outcome and prolong survival, and latterly that the treatment with rituximab may be safely included in a chemotherapy regimen preceding stem cell harvest, high-dose chemotherapy and AT. **Methods.** Between 1997 and 2005, a total of 75 newly diagnosed pts. (44 women, 31 men) with poor-prognosis FL and DLBCL were intensively treated (anthracycline-based therapy) at our department. Chemotherapy with addition of rituximab was administrated in 33 of them (44%). 24 pts. achieved complete remission (CR) and 51 pts. partial remission (PR), mostly with a tumor reduction greater than 50% to 80%. After BEAM conditioning therapy, median of 7.2×10⁹/kg (range, 2.1 - 37.5×10⁹/kg) CD34⁺ peripheral blood stem cells were reinfused. Results. At 100 days following AT, 49 pts. were in CR, 14 pts. in CRu, 7 pts. in PR and 1 pt. relapsed. 4 pts. were shortly after AT and could not be assessed. 15 pts. (20%, 5 with FL, 10 with DLBCL) relapsed/progressed after a median time of 25 months from AT and 5 pts. died from recurrent lymphoma. Only one of the relapsed pts. was treated with rituximab initially (1/3 = 33%), other 14 relapsed pts. were treated with chemotherapy (14/42 = 33%). 60 pts. are still alive in a remission with median follow-up of 34 months (range, 9-117 months) from diagnosis. Estimated 2 years overall survival and event free survival rates are 94% and 87%, respectively.

**Conclusions.** Myeloablative chemotherapy and autologous stem cell transplantation in poor prognosis pts. with advanced DLBCL and FL can lead to long-lasting CR. The standard administration of a front-line immunochemotherapy with rituximab can further improve the quality of remission and prolong event-free and overall survival.

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**1195 CNS LYMPHOMA AND THE USE OF INTRATHecal RITUXIMAB: REPORT OF THREE CASES**

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**Backgrounds.** Central nervous system(CNS) involvement is an adverse prognostic factor for patients with non-Hodgkin’s lymphoma(NHL). Because of the limited passage of rituximab through the blood-brain barrier, intrathecal administration of rituximab has been considered as a possible treatment for CNS lymphoma. Methods. 3 patients with recurrent or persistent CD20⁺ primary parenchymal CNS NHL were treated with myeloablative Chemotherapy and Autologous Hematopoietic Stem Cell Transplantation(ASCT), Three infusions of rituximab at 20 mg dose were given over a 5-week period. One infusion was given in the first, then twice weekly for 4 weeks. All patients received 2 mL of 0.9% saline during 2 minutes. Safety and tolerability were evaluated by clinical evaluation, including neurologic examination, laboratory blood and cerebrospinal fluid (CSF) tests and magnetic resonance imaging (MRI). Results. Intrathecal rituximab administration represents a novel means of treatment of CNS involvement of NHL. Efficacy and safety data are promising, but future trials and follow-up are required to evaluate this route of administration.

**1196 GOOD RESPONSES OF PRIMARY MEDIALIStAL B CELL LYMPHOMA (PMBL) AFTER CHEMIOIMMUNOTHERAPY (CHOP-14 RITUXIMAB) CONSOLIDATED BY BEAM AUTO SCT AND IFRT**


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PMBL is a distinct entity in WHO classification prior described as DLBCL variant. It presents as mediastinal bulky tumor, locally invasive to adjacent mediastinal structures. The bone marrow is involved only in 2% of cases. Relapses tend to be extranodal, including central nervous system, liver, kidneys. Prognosis in PMBL treated by CHOP regimen is poor in most cases resistance of lymphoma cells occurs already during the first line chemotherapy and 5 year overall survival is about 20%. Patients' characteristics: 2005-2005 12 PMBL patients were treated at Hematology Department in Krakow. The medium age patient group was 37.2. In 9 cases the disease was limited to mediastinum (stage I) according Ann Arbor), and subsequent 5 patients had more advanced disease with a spread to vertebral column, lungs or adjacent muscles. B symptoms were present in all cases. None of the patient had bone marrow involvement. The majority of patients had elevated LDH (median 901/ul) and bulky disease at diagnosis (mediastinal mass >20cm) was present in 7 patients, more than 30 cm in 3 patients. IPI was a poor outcome predictor, as it was low (0-1) in 10 cases and intermediate (2-3) in 2 cases. Treatment schedule and results. Patients with PMBL were treated with intensive chemotherapy CHOP-14 Rituximab according GLS (10 patients) or ABCVP chemotherapy according GELA (2 patients). In 8 patients - a good partial response to first line chemother-apy was consolidated by BEAM conditioned by auto SCT. All patients
received IRRT. Although further tumor regression after the transplant was moderate, so far 5-50 months after transplantation PFS is 87%. Four further patient were not transplanted due to denial, ineffective stem cells collection or poor performance status. - 2 of them are in CR , and a 3rd one in a non progressive PR (residual mass ?). Residual masses observed in most of the patients (8/12) at the end of the first line therapy. In the whole group 2 years OS and EFS are 80 and 90% respectively. Conclusion: Intense chemoimmunotherapy does change the prognosis of PMBCL patients although the role of transplant as the first line therapy remains debatable. Effect of radiotherapy is not proven, however similarities between PMBCL and Hodgkin Disease (gene expression analysis, common residual masses at the end of therapy, usually localized disease) make it a tempting therapeutic option.

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NATURAL KILLER/T-CELL LYMPHOMA: A SERIES OF SIX CASES FROM A SINGLE WESTERN INSTITUTION

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Backgrounds. NK/T-cell lymphoma is a rare entity mostly being reported in Asian countries; representing 6% of total of lymphomas. In western countries its incidence is lower and not well described. Neoplastic cells show an angiocentric pattern of infiltration and usually express CD2, CD3e, CD56, TIA and granzyme B. Aim: we described a series of six Caucasian patients diagnosed of NK/T 'cell lymphoma at our institution in Spain in the last 10 years, representing 0.8% of all patients. Patients and Methods. median age was 58 years (from 36 to 72), male sex 5/6 (83%). Five patients presented with involvement of nasal and paranasal cavities and one has a non-nasal type. Patients with nasal involvement presented with nasal obstruction and bleeding. B symptoms were present in 2/6 (33%) patients, High LDH 3/6 (50%). Five of the five patients tested had a positive Epstein-Barr serology. Clinical Ann Arbor staging was: I 3 (50%), II 1 (17%) III 1 (17%) IV 1 (17%) and a modified staging system for paranasal lymphomas was T1 2 (33%) T3 1 (17%) T4 2 (33%). International prognostic index was: low risk 4 (66%), low-intermediate 2 (33%). A modified international index was score 0: one patient, 1 two, 2 two and 3 one patient. 3 out of six patients received chemotherapy including anthracyclines (mostly CHOP) as front line therapy and 2 received radiotherapy and one chemoradiotherapy as a rescue for progression following front-line chemotherapy. Results. Three of the five evaluable patients achieved a complete remission to front line chemotherapy, one achieved a partial response and one of them progressed immediately after chemotherapy (1) and radiotherapy (1). One patient progressed four months after finishing treatment. 2 of the six complete responders relapsed at three and six years after achieving response, all of them with disseminated disease and died as a consequence of lymphoma. Only 2 of the six patients remain alive at the moment. Conclusion: NK/T lymphoma patients diagnosed at our institution presented with clinical features and an aggressive outcome comparable to those described in eastern countries.

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HIGH-DOSE CHEMOTHERAPY WITH TANDEM AUTOLOGOUS TRANSPLANTATION IN RELAPSED/REFRACTORY HODGKIN’S DISEASE - A SINGLE CENTER EXPERIENCE

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Backgrounds. High-dose chemotherapy with autologous stem cell transplantation (auto-HSCT) is commonly used in relapsed/refractory cases of Hodgkin’s disease (HD). Aim: We report the results of tandem auto-HSCT in patients with relapsed/refractory HD. Methods. Thirteen patients were included in this study. The median age was 26 years (range 20-59). Disease status at first auto-HSCT was refractory relapse (n=4) or primary refractory (n=9). Before tandem transplantation all patients received ≥ 2 lines of chemotherapy, one patient received radiotherapy, two relapsed after previous auto HSCT. In eleven patients, only peripheral blood cells were used and in two patients both bone marrow and peripheral stem cells were used. Conditioning regimen with dexmethasone, BCNU, etoposide, cytarabine, melphalan (DexaBEAM) was used for the first transplant and busulfan and cyclophosphamid (BuCy2) in ten patients, and treosulan and cyclophosphamid in three patients for the second transplant. Results. Hematological reconstitution was complete in all patients at both transplants. The median time to neutrophil recovery (absolute neutrophil count ≥0,5G/l) after first and second transplant was respectively: 11 days (range 7-16) and 12 days (range 9-16) and platelet recovery (platelet count ≥20G/l) was respectively: 14 days (range 10-19) and 21 days (range 12-60). After first transplant only neutropenic fever and confirmed bacterial/fungal infections were observed, treated with good results. After second transplant transient congestive heart failure with ventricular arrhythmia and veno occlusive disease was recognized in two patients. One patient (8%) died due to treatment-related toxicity (veno-occlusive disease). In the treosulan group no serious complications was observed. With the median follow-up of 42 months (range 12-71) ten patients are alive (77%), eight are in remission (61%), four patients relapsed (33%). Conclusion. Dose-intensive chemotherapy with tandem transplantation is an option in selected patients with resistant/refractory HD who have poor prognosis and limited treatment opportunity.

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IFOSFAMIDE PLUS VINORELUBE SAVAGE THERAPY FOR REFRACTORY OR RELAPSED HODGKIN Lymphoma: 23 CONSECUTIVE CASES FROM A SINGLE TEAM

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Backgrounds. High dose ifosfamide and vinorelbine is an active regimen in the treatment of refractory or relapsed Hodgkin lymphoma (Bonafide et al, 1998). Aim: To evaluate the efficacy and toxicity of ifosfamide and vinorelbine therapy in a heavily pretreated patient population before or after autologous stem cell transplantation (ASCT). Patients. Twenty-three patients were treated between 2000 Nov and 2006 Feb. The median age at the time of treatment was 28 (18-44) years. The combination was used as first salvage therapy in 7 patients, as second line or relapse, whereas 12 patients received this treatment prior to or without ASCT. All of these 12 patients were treated with ifosfamide and vinorelbine as third or fourth-line therapy. Six of them had primary progressive disease, 3 had early relapse and 3 had late relapse after standard dose therapy. Methods. Ifosfamide (3 g/m2 days 1-4 by continuous infusion) and vinorelbine (25 mg/m2 i.v. days 1 and 4) were administered with G-CSF support and uromitexane uroprotection. The median number of the therapeutic cycles was 2 (range 1-9). Results. The response rate was 65%, with 11 complete (CR 48%) and 4 partial remissions (PR 17%). Of the 15 responding patients, 8 received ASCT, 4 underwent autologous stem cell transplantation with reduced intensity conditioning (NSCT), 2 received further chemotherapy because of progression and 1 had no more therapy and remained in long-term (56 months) complete remission. The regimen was successfully used to mobilize peripheral stem cells in 8 patients (the median number of collected CD34+ cells was 5,05x10⁶/kg), while 3 patients did not mobilize. The main toxic effect was grade IV neutropenia, documented in 96% of cases. Haematologic toxicity was mild. Conclusions. The combination of ifosfamide and vinorelbine proved to be effective to minimize tumor burden before ASCT and NSCT with tolerable toxicity profile. One of our patients
who relapsed following ASCT and had no donor, has remained in continuous complete remission for 56 months.

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COMPARISON OF THE EXPRESSION OF DIFFERENTIATION MARKERS BETWEEN DIFFUSE LARGE B-CELL LYMPHOMA OF NODAL AND EXTRANODAL PRIMARY ORIGIN

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Backgrounds. Diffuse large b-cell lymphoma (DLBCL) is a biologically and clinically heterogeneous lymphoma. DLBCL can be classified into prognostically important subgroups with germinal B-cell (GC) and activated B-cell (non-GC) types with a favorable and an unfavorable prognosis using a CDNA microarray. The expression pattern of differentiation markers CD10, BCL6 and MUM1 by immunohistochemistry (IHC) has been proposed as a surrogate to distinguish GC from non-GC type DLBCL. The purpose of our study was to compare the expression frequencies of a panel of B-cell differentiation markers and proliferation rate in DLBCL according to the primary site, lymph node, or different extranodal organs. Methods. This study included 50 cases of de novo DLBCL; 25 of nodal origin and 25 of primary extranodal origin. Sites of extranodal disease were: stomach (8), spleen (6), testis (4), skin (2), ovary (1), pancreas (1), lung (1) and large bowel (1). All the tissue samples were formalin-fixed paraffin sections obtained by biopsy before chemotherapy. The tumors were subclassified according to WHO classification and evaluated by IHC. To define each case as GC or non-GC type a panel of 3 antigens, CD10, bcl-6 and MUM1 was evaluated following the algorithm reported by Hans CP et al (Blood.2004;103;275). All samples were further analyzed for the expression of bcl-2. Immunoreactivity was judged to be positive if 20% or more tumor cells were stained. The proliferation rate was evaluated by percentage of Ki-67 positive tumor cells. Results. All tumors were CD20 positive. In nodal DLBCL, CD10, bcl-6 and MUM-1 were positive in 17/25 (68%), 15/25 (60%) and 16/25 (64%) cases. Nine cases (36%) were classified into GC type and 16 (64%) into non-GC type. Ten cases (40%) were bcl-2 positive, all of non-GC type. Between extranodal DLBCL, CD10, bcl-6 and MUM-1 were positive in 19/25 (76%), 7/25 (28%) and 9/25 (36%) cases. Sixteen cases (64%) were classified into GC type and 9 (36%) into non-GC type. Eight cases (32%) were bcl-2 positive, all of non-GC type. Non-GC type DLBCL were located in skin (both were leg type DLBCL), testis, ovary, liver and large bowel. The proliferation rate was higher in nodal DLBCL, with a median of 63.2% of tumor cells Ki-67 positive (range from 30% to 80%). In extranodal group the average proliferation rate was 37.6%. The highest proliferation rate (70-80%) was observed in skin and liver DLBCL. Conclusion. In the present series, non-GC type DLBCL, testicular, ovary and liver DLBCL which are known for their aggressive course. The higher frequency of GC type in other extranodal sites implies that they have a more differentiated cellular origin than nodal DLBCL and favorable prognosis.

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T-CELLS DO NOT SUPPORT OSTEOCLASTOGENESIS IN AN IN VITRO MODEL DERIVED FROM NON-HODGKIN LYMPHOMA WITH OSTEOLYTIC LESIONS

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Bone involvement from hematological malignancies other than multiple myeloma (MM) and adult T cell leukemia (ATL) is an uncommon event. It is characterized by osteolytic lesions, whose underlying molecular mechanisms remain ill defined. With regard to non-Hodgkin lymphoma (NHL), osteolytic lesions have been reported to occur in about 5-15% of all cases, rarely as a presenting manifestation of disease. By contrast, MM associated lytic bone disease, observed in 70-80% of MM patients, is more common. In patients with MM, the tumor cells secrete a paracrine soluble factor (RANKL) that is able to induce osteoclasts. In NHL, a similar mechanism may occur, with macrophage-colony stimulating factor (M-CSF), induces osteoclast formation in vitro. RANKL is expressed on malignant cells, osteoclasts, bone marrow stromal cells, CD4+ CD8+ thymocytes, and activated T cells. OPG competes with RANK for binding to RANKL, preventing its osteoclastogenic effect, and can act as a decoy receptor for TNF-related apoptosis-inducing ligand (TRAIL), exerting an anti-apoptotic effect. A linkage between immunoregulation by T cells and bone loss has been found in MM and other bone loss associated diseases, as we demonstrated by means of an in vitro osteoclastogenesis model derived from peripheral blood mononuclear cells (PBMCs) of patients with MM bone disease. In the present study, our aim was to investigate a mode of regulation of bone turnover in lytic involvement from NHL, entailing T cells and secreted factors. We used an in vitro osteoclastogenesis model consisting of unfractonated (and parallel T cell-depleted) PBMC cultures derived from two patients, and established according to the methods we described in Blood, 2004; 104: 3722. The former patient was affected by diffuse large B cell (DLBCL) NHL with osteolytic, the latter had an extranodal DLBCL NHL relapsing at the same site with evidence of disease elsewhere. The controls were represented by five subjects with non-neoplastic disease, without any skeletal involvement. The patients and the controls gave their informed consent. The results showed the occurrence of spontaneous osteoclastogenesis in the unstimulated unfractionated PBMC cultures derived from the patients, but not form the cultures from the controls. The addition of M-CSF and RANKL was not necessary to promote the formation of osteoclasts in the parallel T cell-depleted PBMC cultures. The freshly purified T cells isolated from the patients did not express RANKL, OPG or TRAIL at mRNA as well as at protein level, similarly to the fresh T cells from the controls. We conclude that, in contrast with MM bone disease, T cells of our patients with osteolytic lesions from NHL seem to play no role in the regulation of osteoclastogenesis. Therefore, further investigations are needed in order to better define bone resorption molecular mechanisms in NHL.

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THE PREVALENCE OF ANTI HCV ANTIBODIES IN B-CELL NON-HODGKIN’S LYMPHOMA IN CENTRAL ROMANIA

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Backgrounds. Several epidemiological data suggest the involvement of hepatitis C virus (HCV) in the pathogenesis of some B-cell non-Hodgkin’s lymphomas in areas with high prevalence of HCV infection. Aims. To assess the presence of anti HCV antibodies in a cohort of 54 consecutive patients with B-cell non-Hodgkin’s lymphoma admitted to the Department of Hematology of our center from October 1997 to December 2005 in the Sibiu County (a region in the center of Romania with 464,000 inhabitants). The control group was a cohort of 8,445 blood donors tested in the period of time from 1995 to 2002. Methods. In order to test the presence of anti HCV-antibodies in patients with B-cell NHL a third generation ELISA test was used. The two patients positive for HIV infection were excluded for this study. In the control group a second generation ELISA test was used (from 1995 and, 1999 and a third generation one afterwards. Results. From the 54 patients with B-cell NHL (age between 22-77 years, male/female ratio 1,25) 13 were positive for anti-HCV antibodies (prevalence=24.07%). In the NHL HCV positive patients the male/female ratio was 0,625. In the blood donors control group, 108 were positive for anti-HCV antibodies (prevalence=1,28%). The prevalence of anti HCV antibodies (prevalence=1,28%) in patients with chronic hepatitis) related to chemotherapy. Conclusions. 1. In the county of Sibiu we found a significantly higher prevalence of anti-HCV antibodies in B-cell NHL compared to blood donors control group, indicative of the fact that HCV may be involved in the ethiopathogene-

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Cytomegalovirus Reactivation During Alemtuzumab Therapy for CLL: Safety and Efficacy of Valganciclovir

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Background and Aims: Several study described a variable incidence of cytomegalovirus (CMV) reactivation in patients treated with alemtuzumab. No prospective reports currently provide results of oral valganciclovir as pre-emptive therapy in patients with CMV reactivation during alemtuzumab treatment. We explored the efficacy and safety of oral valganciclovir as a therapy of CMV reactivation and of prophylaxis of CMV disease. Methods: Starting from May 2004, we treated 10 patients (9 males and 1 female; median age 57). Six patients were in partial response after previous chemotherapy regimen containing fludarabine, and 4 were refractory to previous treatment (range 1-7). All patients received alemtuzumab at 10 mg as target dose, 3 times weekly for a prolonged period of 18 weeks. The drug was delivered subcutaneously and, in order to further minimise adverse local therapy-related effects and make the treatment more manageable, were associated with 50 mg of hydrocortisone s.c. for the first two weeks. At baseline all patients had undetectable CMV DNA but were positive by serology. Prophylaxis with oral acyclovir 800 mg bid was given during therapy and for a prolonged period of 18 weeks. CMV reactivation was detected during the treatment with oral valganciclovir. None of the 4 patients showed other episode of CMV reactivation after reintroduction of alemtuzumab. Conclusion: We successfully use valganciclovir in all patients with CMV reactivation. The response was prompt and there was no progression to CMV disease, no relevant clinical toxicity and unnecessary hospitalization for drug administration. Valganciclovir is effective and safe as CMV prophylaxis in CLL patients treated by alemtuzumab, allowing an easy management of a therapy previously difficult to be routinely used.

Mutated or non-mutated? Which Database to Choose When Determining the IgVH Hypermutation Status in Chronic Lymphocytic Leukemia?

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Backgrounds. It has been accepted that the hypermutation status of immunoglobulin heavy chain genes (IgVH) is one of the most important independent prognostic factors in chronic lymphocytic leukemia (CLL). According to the degree of IgVH hypermutation, CLL patients can be stratified into prognostic groups, with favorable or unfavorable prognosis. Aims. Given the impact of IgVH mutation status on clinical setting, it has become highly desirable to standardize the laboratory methodologies used for IgVH mutation status determination. To check the reliability of our laboratory results, we performed an interlaboratory testing, carried out at Homolka Hospital and Hôpital Avicenne. Methods. IgVH hypermutation status was determined in 10 randomly selected CLL patients, according to the Biomed-2 Study protocols. Results. From 10 CLL samples tested, in 9 cases identical results were obtained in both laboratories. In one case, the result was discordant. It turned out that the discrepancy was caused not by a technical obstacle, but by the IgVH database used. This finding prompted us to double-check our cohort of 624 CLL patients, using IgBLAST and IMGT databases. The results showed 7.5% (47/624) discrepancies between both databases. In 21 out of 47 cases, the degree of hypermutation has changed in regard to the database used, resulting in major changes in the prognostic subgroup (Figure 1 below). Other irregularities between both databases were identified, with yet to be determined significance. Conclusions. In the light of...
It is increasingly clear that allogeneic hematopoietic cell transplantation (alloHCT) offers currently the only curative option for chronic lymphocytic leukemia-CLL, but the relatively high transplant related mortality has limited its application. The recent experience following both progressive CLL patients who are in good biological condition. All patients engrafted. Aims. To evaluate the influence of the above mentioned prognostic factors on clinical course of CLL in patients (pts) treated with modern therapy. Patients and methods. Sixty nine pts with B-CLL were included in this study (median age 59,5 years; Binet stage A - 1, B - 41, C - 27; median follow up was 143 mo, median follow up after the start of treat-

predicted data would like to stress the necessity to identify/compile the most comprehensive IgVH database to be used for the determination of IgVH mutation status in CLL.

1205

ALLOTRANSPLANTATION FOR CHRONIC LYMPHOCYTIC LEUKEMIA A SINGLE CENTRE EXPERIENCE IMPLYING ITS APPLICABILITY AND CURATIVE POTENTIAL

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It is increasingly clear that allogeneic hematopoietic cell transplantation (alloHCT) offers currently the only curative option for chronic lymphocytic leukemia-CLL, but the relatively high transplant related mortality has limited its application. The recent experience following both the use of newer first line treatment with purine analogues and less toxic pre-transplant preparative regimens appeal for wider trials evaluating alloHCT early in the CLL course in younger patients. Materials. Ten patients (F/M=5/5), median age 44.5y (36-55), time from diagnosis to alloHCT 3 years (1-7.5). After diagnosis patients were treated using 1-5 different chemotherapy regimens, all obtained purine analogues and all displayed treatment resistant and progressive course. Other treatments included radiotherapy (n=2), anti-CD20 MoAb (n=2), anti-CD52 MoAb (n=1), rituximab (n=1), and radiotherapy (n=1). The disease status at alloHCT was as follows: CR n=4, PR n=3, NR n=3. alloHCT characteristics: HLA matched Sibling Donor HCT (n=8), HLA single allele mismatched SibeDHTC (n=1), matched Unrelated Donor HCT (n=1). Stem cell source for SibD transplant: bone marrow - 2, peripheral blood -2 (two positive products of CD34+ in CD34 cells and CD3 cell add back), BM+PB -1, for URD-HCT ‘bone marrow in 1 pt. Conditioning: myeloblastic Ctx+TBI: n=2, Ctx+TBI+alemtuzumab: n=1; reduced intensity conditioning: Ctx+TBI+alemtuzumab: n=1; reduced intensity: alectumuzumab (20 mg/kg)+fludarabine (30 mg/m2 2x5)+melphalan (140 mg/m2); n=7. The number of transplanted cells: nucleated cells 4.25x10^9 (0.045-12); CD34+ cells 4.3x10^3 (1.5-9.6), CD3+cells 5.5x10^3 (15-514) k/recipients body weight. All transplantations were performed in intensive care, sterile HEPA units. GVHD prophylaxis consisted of cyclosporine A and methotrexate. Results. All patients engrafted. Hematopoietic recovery was as follows: granulocytes to 0.5 G/l -22 d (11-55) ; PLT to 50 G/l/24d (13-40). One patient died on day 92 after transplantation of pulmonary Aspergillosis and hepatitis after LPD due to EBV infection transmitted from the donor. The remaining 9 patients achieved CR after transplantation. All 3 patients after myeloablative conditioning acquired full donor chimerism. Among RIC conditioned patients at 6 months 2 displayed full donor chimerism, 3 mixed chimerism and one presented autologous recovery. Acute GVHD grade I was observed in 3/10 patients, limited cGVHD in 3 patients and extensive cGVHD in 2. Six patients developed CMV reactivation, one VZV, and one HBV. Two patients (both after ablative conditioning) died due to late complications: on day 180 (cGVHD with obstructive bronchiolitis) and on day 720 (chronic hepatitis). No patient relapsed with CLL suggesting efficacy of the mechanism. At 53 months after transplantation the probability of OS and DFS equals 60% with median observation time of 13 months (7-53). This observation compares well with recent other data (Toze CL et al 2005; 5y OS 59%) and suggests that allotransplantation offers an effective treatment with curative potential for progressive CLL patients who are in good biological condition.

1206

SIGNIFICANCE OF SOME FACTORS IN THE ERA OF MODERN CLL THERAPY

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Background. Expression of CD38, high level of Bcl-2 and CD95 expression of cases consecutively referred to our center because of persistent lymphocytic leukemia, i.e. by the dominating expression of CD38 (RR=0,059) were the most unfavourable factors. Median PFS was not achieved in pts with any UPF combinations without CD38 expression where as in pts with all 4 UPF it was only 20 mo. Conclusion. Modern therapy with FC and RFC allows overcome the negative influence of high level of B2-microglobulin and Bcl and lack of CD95 expression. CD38 expression retains its unfavourable significance.
lymphocytosis, focusing in particular on the differential diagnosis of clonal B-cell lymphocytoses. Methods. Between January 2003 and December 2004, we evaluated 373 consecutive patients (M/F: 190/183) with an absolute (i.e. >5000/m3) (81%) or relative (19%) lymphocytosis. Median age was 68 years (range: 19-91). Clinical features, lymphocytocyte morphology, immunophenotype, BM and/or lymphnode histopathology were reviewed. Morphology and immunophenotype were independently reviewed by three experts. Immunophenotype was performed on fresh whole blood samples using four-color immunostaining with a panel of B- and T-cell markers, a FACSCalibur flow cytometer (BD Biosciences) and the CellQuest software (BD Biosciences). T-cell clonality was assessed by PCR analysis of the T-cell receptor (TCR) γ chain variable regions and using the TCR V8 Kit (Beckman-Coulter Immunotech, Marseille, France) for the TCRβ chain families repertoire. Histopathology evaluation of BM and/or lymphnode was performed in cases whose PB morphology and immunophenotype suggested a likely diagnosis of B/T non-Hodgkin lymphoma (NHL) or could not distinguish between B-NHL/chronic lymphocytic leukemia (CLL). Results. A B-lineage lymphocytosis was recorded in 81% cases (n=301; 241 CD5+ and 60 CD5-), a T-lineage in 14% (n=52) and a normal lymphocyte pattern in 5% (n=20). In terms of PB morphologic/immunophenotypic criteria, among clonal B-lymphocytoses, 44.5% (n=134) had features of CLL, 44.5% (n=134) were B-NHL, 10% (n=30) were provisionally defined as B-NHL/CLL for intermediate features, 1% (n=3) were hairy cell leukemia (HCL). Of 107 CD5- cases not fulfilling the standard diagnosis of CLL, 59 underwent BM and/or lymphnode biopsy; 41% were diagnosed as B-NHL with leukemic spillover (5 marginal zone lymphomas (MZL), 4 mantle cell lymphomas, 2 follicular lymphomas (FL), 2 lymphoplasmacytic lymphomas (LPL), 11 unspecified low grade B-NHL) and 59% with CL. Even among CD5-CD25+ cases not fulfilling the standard diagnosis of CLL, 25% (11/43) proved to be leukemic B-NHL at histopathology evaluation. Of 60 CD5- cases, 37 underwent a biopsy; the final diagnosis was MZL in 21 cases, FL in 4, LPL in 3, HCL in 2, unspecified low grade B-NHL in 7. In CD5- B-NHL and CD5- B-NHL, the expression of CD38 (p<0.001) and adhesion molecules (CD11a, CD18) (p<0.001) were significantly higher than in CLL, as well as the presence of superficial adenopathy (p<0.001), splenomegaly (p<0.001), thrombocytopenia (p<0.05) and raised LDH (p<0.001). Among T-NK lymphocytoses, 48 cases showed a T-LGL expansion (reactive in 21, clonal in 22), 2 NR-LGL, 3 T-NHL, 4 unclassified T-cell lymphoproliferative disorders. Conclusions. Our study highlights the frequency of various B- and T-cell neoplasms presenting as lymphocytosis and the value of lymphocyte morphology, immunophenotype and histopathology in identifying and subclassifying low grade B-NHL with leukemic presentation. These observations have prognostic-therapeutic implications.

1209
THE USE AND CORRELATION WITH FLOW CYTOMETRY OF FLUORESCENT MOLECULAR BEACONS IN REAL TIME PCR OF IGH GENE RARRAYEMENTS FOR EVALUATION OF MINIMAL RESIDUAL DISEASE IN MULTIPLE MYELOMA

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At present, the prognostic value of the amount of residual tumor cells in PB, BM and/or stem cell harvests and its changes over time is still not clear. Also the advent of new therapeutic approaches to multiple myeloma made necessary the introduction of novel methods for detection of minimal residual disease. Among others approaches residual disease can be detected by using flow cytometry. The aim of the present study was to evaluate a real time PCR test for the IGH gene using allele-specific molecular beacons and fluorescence probes to quantify residual disease and also correlate flow cytometric detection of plasma cells in MM patients during followup after treatment with high dose of chemotherapy or standard chemotherapy. After clinical diagnosis of 17 MM patients, the CDR1, CDR2 and CDR3 regions of the IGH gene were analysed and sequenced to identify its clonal nature. Unique sequences of the clonal IGH rearrangement were used to design specific molecular beacon probes for each MM patient. We have also examined the co-expression of CD19, CD38, CD45, CD56, and CD138 molecules in cells of bone marrow aspirates in patients with multiple myeloma by flow cytometry. Results: disease had been accepted of whom plasma cell infiltration ratio was over 10% in bone marrow and also of whom labeled by CD38 and CD138 by FCM. The detection of the MRD was positive in 13 patients by RT-PCR, respectively. The infiltration ratio was correlated with CD138 expression (r=0.009) and RT-PCR detection of plasma cells (p=0.006) and also significant correlation had been found between RT-PCR detection and CD138 expression respectively. No any correlation was found between other surface antigens (CD38, CD45, CD56). Our results indicated that real time PCR with specific molecular beacons provides a feasible, accurate and reproducible method for the determination of minimal residual disease in MM. By FCM only CD138 expression may have been used as disease marker in addition of the RTPCR detection.

1210
BORTEZOMIB IN COMBINATION WITH HIGH-DOSE DEXAMETHASONE FOR RELAPSED MULTIPLE MYELOMA

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Backgrounds. Single agent bortezomib treatment yields partial responses (PR) in 24% of patients with relapsed, refractory multiple myeloma (MM) and 38% in patients who had received 1 - 3 previous therapies. Dexamethasone (DEX) increases bortezomib anti-myeloma activity. The present study was initiated to study bortezomib in combination with DEX. Patients and Methods. 82 patients (pts) with advanced MM were scheduled to receive bortezomib 1.3 mg/m2 IV days 1, 4, 8, and 11 q 3 weeks for 8 cycles in combination with DEX 40 mg IV OR PO on the day of bortezomib injection and the day after. Patient characteristics were median age 66 years; β-2-Microglobulin> 3,0 mg/l, 64%; median number of prior therapies 3 (range 2 - 6), and 38% of patients had relapsed after high-dose melphalan. EBMT criteria were used for response definition.

Results. Four pts (13%) achieved a complete response (IF negative), five pts (16%) a complete response (IF positive), 18 pts (38%) a PR, and 0 (0%) a minor response (MR) resulting in an overall response rate (ORR) of 69%. Median time to disease progression (TtPD) was 189 days (6,3 m). After a median follow-up of 18 months, median overall survival was 294 days (10 m). The median number of cycles administered was 4 (range 1-8). Grade 4 neutropenia was observed in one patient (3%), grade 4 thrombocytopenia in 6 pts (19%), without any thrombocytopenic bleeding. Grade 3/4 non-hematologic toxicities requiring dose or schedule modifications were peripheral neuropathy (6%), fatigue (4%), herpes zoster (9%), and cutaneus events (3%). Conclusions. Bortezomib in combination with DEX is a highly active regimen without increased toxicity as compared to a single agent treatment with bortezomib.

1211
BONDRONAT IN THE TREATMENT OF BONE LESIONS IN MULTIPLE MYELOMA

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Backgrounds. Multiple myeloma (MM) is a malignant disease characterized by skeletal involvement. Osteolytic bone lesions from MM are associated with skeletal complications such as bone pain and pathologic fractures which have a negative impact on quality of life. Bisphosphonates have been shown to decrease the progression of osteolytic lesions,
bone pain and fractures. Aims. Aim of this study has been to evaluate the efficiency of ibandronate (Bondronat, F Hoffmann-La Roche) on the course of bone lesions in myeloma patients and also its safety particularly concerning renal function. Methods. We analysed a group of 28 patients in clinical stage IIIA or IIIB (median age 59.9 years, range 42-77, male: female ratio 13:15) who were currently treated, independently from the adopted chemotherapy, with Bondronat given as an IV infusion every four weeks for up to 31.7% at 36 infusions. The site of ONJ was maxilla in three patients and mandible in four. All had received treatment with chemotherapy, including creatinine, calcium, phosphorus, and AP. were performed. Markers of tubular damage (NAG, AAP, γ-GT and β-M) were measured in urine before and after Bondronat administration. Results. Clinical improvement of skeletal pain was observed in 23 pts (82%): 12 pts (48%) had a complete pain relief with no more necessity for analgesic-drug use, and 11 pts (59%) had a minor effect, while 5 pts (18%) had no improvement. Nine patients had pathologic fractures at baseline (8 were on vertebral bodies and 1 on ribs) and all of them underwent radiotherapy. During the observed period we didn’t find any new pathologic fracture. There were no significant adverse effects associated with the administration of Bondronat. Twenty-one pts (75%) had normal renal function at baseline and the rest had various degrees of renal insufficiency. No clinically relevant changes in serum creatinine occurred even in patients with existing renal impairment. Transient hypocalcaemia was detected in 3 pts (10%). The levels of NAG, AAP, γ-GT and β-2M were similar before and after administration of Bondronat. Conclusions. The treatment with Bondronat has reduced skeletal morbidity and fistulae in our group of myeloma patients and we didn’t find any signs of acute renal toxicity in the observed period.

1212 OSTEONECROSIS OF THE JAW IN PATIENTS WITH MULTIPLE MYELOMA DURING AND AFTER TREATMENT WITH ZOLEDRONIC ACID

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Backgrounds. An increase of the incidence of osteonecrosis of the jaw (ONJ) in subjects with multiple myeloma (MM) receiving zolendronic acid has been reported in recent years. Aims. Based in these reports, we analyze the incidence, the clinical features, and the factors associated with the development of ONJ in patients with MM treated with zolendronic acid. Methods. Forty-four patients diagnosed with MM and treated with zolendronic acid, alone or after pamidronate, between August 1996 and March 2006 were enrolled in this study. Demographic data, predisposing factors (including dental extractions and oral surgery), the antimyeloma therapy received and the number of courses of bisphosphonates were recorded and compared with the results of the published series. The main characteristics of the seven patients with ONJ, including risk factors of the complication, clinical and physical examinations data, diagnostic methods and treatment established were reported. Results. The overall incidence of ONJ was 7 out of 64 patients (10.9%). The ONJ has been associated with a recent oral surgical procedure (p=0.0001) and with the prior presence of dental or periodontal pathology (p=0.007). There was no association among the emergence of ONJ with age (p=0.536), sex (p=0.547), type of paraprotein (p=0.778), presence of lytic bony lesions (p=0.667), therapy with dexamethasone (p=0.128), thalidomide (p=0.564), auto-stem cell transplantation (p=0.17) or receiving >3 different courses of oncologic treatments. The means of infusions of bisphosphonates (zolendronic plus pamidronate) and of zolendronic acid before onset of osteonecrosis (SD) were respectively 38.1 (14.7) and 30 (7.0) in contrast to 22.5 (16.4) of bisphosphonates and 19.5 (11.8) cycles of zolendronate (p=0.05) in the patients who didn’t present at this complication. The cumulative risk of ONJ increased from 6.7% after 20 treatments with zolendronic acid up to 31.7% at 36 infusions. The site of ONJ was maxilla in three patients and mandible in four. All had received treatment with chemotherapy, dexamethasone, thalidomide or auto-stem cell transplantation. Three patients were receiving pamidronate before zolendronic acid. The clinical and examination data were pain in all the cases associated to dental ulcer and local infection in six patients. The diagnosis was confirmed in all the cases with panoramic radiology or CT scan of the jaw, and a biopsy was obtained to exclude metastatic disease in four cases. Zoledronic acid infusions were discontinued in six patients: 3 at the time of development of new lesions of ONJ. Three patients exhibited osteonecrotic lesions of the jaw after discontinuing zolendronic acid several months before. All patients received treatment with corticosteroids, antibiotics and surgical debridement. The follow-up after the diagnosis of ONJ was at least six months. Conclusions. The ONJ in patients with MM who underwent dental or oral surgery appears to be associated with long term exposure to zolendronic acid. A previous dental pathology and the time of exposure to zoledronic acid are main factors in the development of the ONJ. The long-lasting bone effect of bisphosphonate could explain the appearance of osteonecrotic lesions after discontinuing treatment with bisphosphonate.

1213 RE-TREATMENT WITH BORTEZOMIB IN MULTIPLE MYELOMA

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Background and Aims. Bortezomib is an effective agent for multiple myeloma, currently licensed for the treatment of relapsed/refractory disease after first line therapy. There are, however, few reports about the use of bortezomib as re-treatment (re-challenge) in myeloma patients who have previously received the same drug for their disease. The clinical outcome of five patients with these characteristics is reported. Methods. Five patients were re-treated with bortezomib alone or in combination; three were male and two female, with an age ranging from 45 to 66. All patients had a prior salvage therapy with bortezomib or bortezomib plus dexamethasone, after 2 to 4 lines of other chemotherapies, including autologous stem cell transplantation and thalidomide. All patients achieved at least a partial response (reduction of M-component >50%) after the first treatment with bortezomib and relapsed after 3 to 19 months. Results. Bortezomib was re-administered at the standard dose of 1.3 mg/m² IV, days 1, 4, 8, 11, q2wks in four patients; dexamethasone (20 mg/d for 2 days after each infusion of bortezomib) was added in 3 out of 4 patients. Severe hematological or extra-hematological toxicities did not occur, but dose reduction or temporary interruption of the treatment were occasionally required, mainly due to moderate thrombocytopenia, neuropathy and skin rashes. After 4-8 cycles, three patients achieved a partial response, with reduction of M-component >50% and concomitant consistent decrease of marrow plasma cell infiltration. Duration of second response to bortezomib ranged from 3 to 8 months. In one patient a stabilization of the disease was obtained. In the fifth patient bortezomib was employed at the dose of 1.3 mg/m² days 1 and 4 in combination with melphalan (100 mg/m² e.v.), thalidomide (100 mg/d for 5 days) and dexamethasone (40 mg e.v. for 4 days), who had the disease regimen (MVTGR) for a further autologous stem cell transplantation. An impressive, rapid complete response with negative serum immunofixation (M-component was 6.1 g/dl before transplant) occurred. This response had a brief length and the patient relapsed after 3 months. The same regimen was given once again and the complete response was achieved in a few days. The patient, however, died of interstitial pneumonia during the aplastic phase of transplant. Conclusions. Our data, although limited, suggest that re-treatment with bortezomib of myeloma patients, who experienced a clinical benefit after the first treatment, is feasible and may induce a new significant response.

1214 A SINGLE CENTER REPORT ON AUTOLOGOUS STEM CELL TRANSPLANTATION FOR PATIENTS WITH MULTIPLE MYELOMA

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Autologous stem cell transplantation (ASCT) is a recommended treatment option for patients with advanced multiple myeloma (MM). From Oct. 1996 to Aug. 2005 we performed 121 ASCT in 71 MM-patients (age 56 (median: 57-68) years, female: male, 40:31). Thirtythree patients (pts) underwent ASCT with a single course (20 pts. between 1996-1999 and 7 pts. thereafter due to ineligibility for a second transplant (e.g. late infections (2), toxic side effects: cardiotoxicity (2), neurotoxicity (1), dermatitis (1), sMDS (1)). Thirtyeighty patients underwent multiple cours-
es of transplantsations (26 double and 12 triple ASCT). No significant differences between both groups were seen according to age, sex or stage of disease at the time of ASCT, but more patients who relapsed after conventional treatment (15 vs. 5 pts) were included in the single than in the multiple ASCT group. Conditioning chemotherapy consisted of Melphalan 200 mg/m² for single and double ASCT and 100 mg/m² for triple ASCT. One patient with severe stem cell hematological recovery (median time to PMN>0.5 G/L : 11(8-13) days and to PLT > 50 G/L : 15 (8-55) days) did not differ between the first or the following transplantsations. All patients but one in each group responded to transplantation. In the single ASCT group 1 treatment related death occurred and in the multiple ASCT group 1 pt. had progressive disease shortly after tandem transplantation. The complete remission rate (< 5% plasma cells in bone marrow and disappearance of paraproteinemia and/or paraproteinuria) was 42% and did not differ between the two groups (14/35 pts. vs 15/38 pts). Although the relapse rate is higher in the single ASCT group (22/35 pts.) than in the multiple ASCT group (15/38 pts.) no significant difference could be seen in median progression free survival (25 vs. 28 months) and the median overall survival (69 months vs. not reached yet), caused by a longer observation time for single ASCT (median 42 (1-147) months) than for multiple ASCT (median 25 (5-71) months). Autologous transplantation is a tolerable treatment option even for older patients with multiple myeloma. In 85% of the patients provided with transplantation all courses of ASCT could be performed. Due to heterogeneity of the patients and the different observation periods no final conclusions can be drawn concerning the outcome of the transplantation.

1215 SERUM INTERLEUKIN-17 AND ITS RELATIONSHIP WITH ANGIOGENIC FACTORS IN MULTIPLE MYELOMA

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Background. Interleukin-17 (IL-17) is a CD4 T-cell derived mediator of angiogenesis that stimulates vascular endothelial cell migration and regulates production of a variety of proangiogenic factors, such as tumor necrosis factor-α (TNF-α) and vascular endothelial-cell growth factor (VEGF). Angiogenesis is implicated in the progression of multiple myeloma (MM). Overexpression of two potent inducers of angiogenesis - TNF-α and VEGF - has been found in MM cell lines and in the serum of patients with the disease. In MM, bone marrow angiogenesis parallels tumor progression, and is angiogenesis surrogate for the disease burden in myeloma patients, indicating a regulation of disease activity. The levels of these cytokines were measured in serum samples from 12 healthy volunteers, 20 patients with multiple myeloma, and 15 healthy persons, age and sex-matched to the myeloma patients. Serum samples from 12 persons, age and sex-matched to the myeloma patients were measured by solid-phase sandwich enzyme-linked immunosorbent assay (ELISA) using monoclonal human antibodies against IL-17, VEGF, and TNF-α from commercially available test kits (Quantikine® and R&D Systems Inc. Minneapolis MN, USA). Blood vessels were highlighted by immunohistologic techniques with a monoclonal antibody to CD34. Microvessel density (MVD) was assessed in all patients biopsies and 15 non-tumor control biopsies. Due to heterogeneity of the patients and the different observation periods no final conclusions can be drawn concerning the outcome of the transplantation.

1216 EARLY DEATH AND MULTIPLE MYELOMA. EXPERIENCE AT A SINGLE INSTITUTION

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Background. Early Mortality after diagnosis of multiple myeloma (MM) is often attributed to combined effects of active disease and comorbid factors. Symptomatic myeloma causes anemia (40%), thrombocytopenia (14%), and neutropenia (6%) at diagnosis. Skeletal disease (70%) reduces mobility, impairs ventilation, and may result in hypercalcemia. Aims. The aim of our study was to assess early mortality associated with infectious complications in patients with newly diagnosed multiple myeloma. Material and methods. We enrolled all patients who fulfilled entire criteria for multiple myeloma between January 1995 and December 2005. The present study is a retrospective, descriptive and observational one. Early mortality was calculated from date of entry onto the trial to date of death or date last seen, as appropriate, and was defined as death within 60 days. Infectious diseases were confirmed clinically or microbiologically. Secondary endpoint was to assess the response rate. It was estimated based on the best response to therapy for each patient during the course of treatment. Statistical analyses were performed using SPSS version 13.0. Fisher’s Exact test was used to evaluate the statistical significance of associations in two-way contingency tables. A p value <0.05 was considered as statistically significant. Results. Between January 1995 and December 2005, we treated 122 patients with multiple myeloma, 67 patients received VAD (54.9%), 85 patients received thalidomide and dexamethasone (28.7%) and 20 patients received melphalan/prednisone (MP, 16%). The frequency of death was in CR 22.8%, VGR/PR 40% (VGR: 22.8, PR: 17.2%), and VAD (24%) (CR 22.8%, VGR: 30%, VAD: 37%). There was a significant correlation between early death (ED) and evidence of hematopoietic dysfunction as evidenced by anemia (p=0.001), thrombocytopenia (p<0.0001), neutropenia (p=0.04), and lymphocytopenia (p=0.007). Renal function was impaired in twice as many of the early death patients with higher presentation serum creatinine and urea (p<0.005). Renal failure was contributory to 4/12 early deaths. Bacterial infection directly caused 11/12 early deaths (91%). Specifically pneumonia occurred in 42 (50%) of 85 bacterial infections (122 patients), and 11/12 patients in the ED group. Generalized sepsis occurred in 18 (15%) of 136 bacterial infections, and other infections occurred in 52 (58%) urinary tract infections and 24 patients (18%) (eg. osteomyelitis, peritonitis and meningitis). Conclusions. This study describes the complications and related mortality that occur soon after diagnosis of myeloma is made. Measures to prevent infectious complications has been described previously. In addition, reduction of renal toxicity also has to be mandatory.

1217 INTERNATIONAL STAGING SYSTEM FOR MULTIPLE MYELOMA IN A MEXICAN POPULATION

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Background. Studies conducted in the 1960’s and early 1970’s identified a number of clinical and laboratory parameters that are independent predictors of survival duration. In 1975, Durie and Salmon introduced a staging system that predicted myeloma cell tumor burden. Factors in the DS classification included the level and type of paraprotein, hemoglobin, calcium level, and number of bone lesions. Creatinine level (Substage A: serum creatinine <2 mg/dl; and substage B: serum creatinine >2 mg/dl) further defined lower versus higher risk patients in each tumor mass stages. Recently Greipp et al, reported the International Staging System (ISS) as a simple and reliable staging system for multiple myeloma. The ISS system was further validated by demonstrating effectiveness in patients in North America, Europe and Asia but not in Central America. Aims. The main objective of our study was to assess the effectiveness of ISS in a mexican population. We also reported the outcomes in terms of overall response according to each group of treatment and ISS was compared with the DS staging system.
Patients, material and methods. We enrolled all patients who fulfilled the entry criteria for multiple myeloma between January 1995 and December 2005. The present study is a retrospective, descriptive, longitudinal and observational one. All patients had survival status and date of last follow-up recorded within 6 months of the data analysis. At the time of analysis, 54.1% of patients had died. Statistical analysis: Univariate and multivariate survival analysis. The variables are ranked by hazard ratio, with all being significant at the p<0.001 level. Fisher's Exact test was used to evaluate the statistical significance of associations in two-way contingency tables. A p value <0.05 was considered as statistically significant. Results. One hundred and twenty two newly diagnosed multiple myeloma patients were evaluated, 76 (62.3%) male and 46 (37.7%) female, aged 46-85 (median 64). Compared with the DS classification, ISS provides a simple reliable classification of patients. ISS stage I is underrepresented, maybe because stage I patients usually are asymptomatic or not included in clinical trials. B2 microglobulin greater than 5.5 mg/dl appeared to be the most highly statistically significant result (p=0.005). Median survivals were as follows: I, 74 months; II, 48 months and 3, 20 months (p=0.0002 for differences). We also evaluated outcomes in terms of survival; creatinine >2 mg/dl, p=0.03, platelet count less than 130,000, p<0.005, CRP >6 mg/L, p<0.003, albumin <35 g/L (p<0.005) and cytogenetics abnormalities such as del 13, located in worst overall survival. We reported an increased mortality in those patients with 1q/2 abnormalities or deletion 13 (p=0.08). Conclusion. We found that B2 microglobulin higher than 5.5 mg/L is the best cut-off to discriminate survival in newly diagnosed MM patients. Based on this result the following question is mandatory: why are serum B2MG and serum albumin such powerful prognostic factors? Serum B2MG reflects not only tumor mass and renal function but also other as yet unknown parameters, possibly including immune function.

1219

DIFFERENCE BETWEEN MALE AND FEMALE PATIENTS WITH MULTIPLE MYELOMA ON LIPID PROFILE

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Purpose: The aim of this study was to investigate the difference between male and female patients with multiple myeloma on lipid profile. Material and Methods. 77 inpatients with multiple myeloma, aged 73±7, 42 females (F) and 35 males (M) were studied. Serum protein electrophoresis and immunoelectrophoresis (IgG, IgA, IgM, and IgE) and plasma lipid levels as Total Cholesterol (TC), Triglycerides (TG), HDL and LDL, from all patients were measured and compared between females and males. All patients belong to our Internal Medicine Clinic and results were analyzed in the same laboratory. Results: Electrophoresis (%): Albumin 45.6±9.5, 50.6±2.62 (M) - 39.8±9.1 (F), p=0.21, β-1 globulin 5.3±1.2, 5.7±1.8 (M) - 3.2±1.2 (F), p=0.02, β-2 globulin 10.7±2.3, 14.2±2.9 (M) - 8.8±3.7 (F), p=0.57, β globulin 13.1±3.3, 10.6±3.6 (M) - 14.4±9.8 (F), p=0.16, γ globulin 29.8±16.4, 21.6±11.6 (M) - 34.2±17.3 (F), p=0.07, Immunoelectrophoresis (mg/dl): IgG 2439±2636, 2999±1959 (M) - 2105±2260 (F), p=0.04, IgA 998±1489, 485±784 (M) - 1306±1730 (F), p=0.003, IgM 59±66, 66±71 (M) - 60±65 (F), p=0.63, IgE 284±574, 61±266 (M) - 507±962 (F), p=0.02, Ligh chains (g/L): Igκ - 3.8±10.0, 12.1±10.5 (M) - 1.3±1.5 (F), p=0.004, Igλ - 2.9±3.7, 1.2±2.9 (M) - 5.9±4.3 (F), p=0.02, Lipid profile (mg/dl): TC 173±54, 147±81 (M) - 183±55 (F), p=0.000, TG 154±69, 108±62 (M) - 175±62 (F), p=0.04, HDL 42±12, 33±17 (M) - 45±9 (F), p=0.01 and LDL 95±42, 73±57 (M) - 102±52 (F), p=0.02. Conclusion: The study shows that in patients with multiple myeloma the TC, HDL and LDL are increased statistically significant in females, while the level TG between female and male have not statistically significant.

1220

VELCADE AN ACTIVE AGENT FOR MULTIPLE MYELOMA PATIENTS. EXPERIENCE OF A SINGLE CENTER

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Velcade (PS-341, Bortezemib), a proteasome inhibitor, has been shown to be efficient in the treatment of relapsed and refractory Multiple Myeloma (MM) and approved for this indication. Aims. We report the results of a nearly 3-year experience regarding the use of this drug in this subset of patients followed in our center. Between April 2003 and February 2006 we treated 27 patients with MM. Patient population consists of 15 males and 12 females, with a median age of 55 years (range 32-76). 17 were IgG, 9 IgA, 1 light chain. All patients were in stage III of disease with a median time of observation (from diagnosis
To Velcade therapy of 54 months (range 11-120), pretreated at least with two lines of therapies and were refractory or in relapse after the last treatment. Thirteen patients had undertaken autologous transplantation and 2 allogeneic one, with a median number of previous therapeutic lines of 2 (range 1-5). Velcade 1.3 mg/m² was administered on day 1, 4, 8 and 11 of a 21-day treatment cycle for 8 cycles according to the tolerability and patient's daily hospital regime. A median of 6 cycles (range 2-8) were administered to the overall population. Thirteen patients concluded their program and 14 discontinued the treatment: 1 because received allogenic stem cell transplantation, 8 for adverse events and 5 for progression of disease. In this heavily pretreated population our primary end point was to obtain a decline in Monoclonal Component (MC) of at least 25%. All patients but 2 were considered evaluable for response because treated at least with 3 cycles of therapy. Thirteen patients responded to treatment: 7 (28%) achieved a reduction of MC level >75%, 4 (16%) <75% and > 50% and 2 (8%) <50% and > 25%. Twelve (48%) showed no response. The median number of cycles to achieve a response was 3 (range 1-8). After a median time of observation of 28 months (range 5-34) the median duration of response was 7.5 months with 5 patients still in response, 8 relapsed and 4 of them died for progression of disease. Among the 12 (48%) not responding patients 3 died. The majority of adverse events, resolved with the discontinuation of treatment, were nausea, vomiting, diarrhea, fatigue, thrombocytopenia, infection, peripheral neuropathy. A very poor prognosis of our patients, this study adds further evidence concerning the efficacy of this new drug. Velcade can be considered an effective anti-myeloma drug even though its toxicity must be taken into account in designing new clinical trials.

**1221**

**BORTEZOMIB WITH OR WITHOUT DEXAMETHASONE IN HEAVILY PRETREATED MYELOMA PATIENTS: PRELIMINARY SAFETY AND ACTIVITY PROFILE FROM A SINGLE CENTRE**


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**Background/Aims.** Patients with multiple myeloma respond to front-line chemotherapy but relapse is virtually inevitable and response duration decreased after salvage regimens. Bortezomib, a proteasome inhibitor, is approved for the treatment of relapsed myeloma, while addition of dexamethasone may result in enhanced tumour control. We evaluated the activity and safety of bortezomib with or without dexamethasone in 20 pretreated myeloma patients. Methods. 20 heavily pretreated myeloma patients (median number of previous therapies 4), with a median age of 73 years (range 54-82), treated myeloma patients (median number of previous therapies 4), with a median age of 73 years (range 54-82), received bortezomib 1.3 mg/m² intravenously on days 1, 4, 8 and 11 of a 21-day cycle for eight cycles. When it was combined with dexamethasone, this was given on days 1, 2, 4, 5, 8 and 9 of a 21-day cycle for eight cycles. The baseline serum concentration of monoclonal protein (mean): in first group from 5.5 g/dL to 25 g/dL, in second group from 11 g/dL to 30 g/dL. The median number of cycles administered was 4 (range 1-6). The toxic event most frequently responsible for therapy withdrawal was grade 3 peripheral neuropathy. Three cases of grade 3 peripheral neuropathy were observed. Thrombocytopenia was the most frequent adverse event (3 cases of grade 4 and 9 of grade 3) but no severe hemorrhagic episode took place. Three patients had episodes of paralytic ileus leading to treatment discontinuation. With a median follow-up of 11 months, 17 patients had a response (1 CR - 16 FR), while three patients had refractory disease. 14 of 20 patients are alive and 7 out of 20 in remission. The median time to progression was 12 months and the 1-year progression free survival was 54% (95% CI: 38-65.5 months). For the monotherapy group the median time to progression was 10 months whereas for the combination dexamethasone - bortezomib 12 months, a difference not significant (Log rank 2-sided p=0.72). Among responders the median duration of response was 7 months (range 3-12). For the monotherapy and combined treatment groups the median duration of response was 5 and 7.5 months respectively (Student t-test p=0.65). The 1-year overall survival is 80% with the median overall survival not reached yet. Conclusion. We report evidence of satisfactory activity of bortezomib/dexamethasone in this group of 20 heavily pretreated patients with advanced myeloma. Peripheral neuropathy was unexpectedly a major problem in a patient cohort pretreated with nerve-damaging therapies such as VAD and thalidomide. Neurologic toxicity caused reduction of dose-intensity and bortezomib discontinuation, factors abrogating the overall antitumour effect. Research efforts towards modulation of neurotoxicity and optimisation of bortezomib schedules may pave the way for enhanced myeloma control.

**1222**

**LEUKOCYTE ALKALINE PHOSPHATASE SCORE IN MULTIPLE MYELOMA; CORRELATION WITH G-CSF, IL-6 AND TNF-α**

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**Backgrounds.** The leukocyte alkaline phosphatase (LAP) score has been reported to be elevated in patients with multiple myeloma (MM), but its clinical significance has not been clarified. Some authors reported that interleukin-6, IL-6 and G-CSF genes were co-expressed in most patients with MM. Aim: In the present study, we determined the LAP scores of peripheral blood neutrophils and the serum levels of G-CSF, IL-6 and tumor necrosis factor-α (TNF-α) in patients with MM at diagnosis, and healthy controls and made the relative correlations. Material and Methods. We examined 25 patients with MM, age 62±10, at diagnosis and 10 normal subjects, age 45±5. The LAP score was examined by using naphthol AS-BI phosphate (Sigma Chemical St.Louis, MO). The serum levels of G-CSF, IL-6 and TNF-α were measured by a sandwich enzyme immunoassay test (R&D Systems, Minneapolis, MN, USA) and measured in a Microplate Reader Sirio-Brio™ (Radim Group). Results. The mean LAP scores of patients with MM vs control subjects were 295±58 and 187±46 respectively. The mean value of LAP in MM patients is significantly higher (p<0.001). The serum G-CSF levels of the patients with MM vs those of controls were 15.2±12.4 pg/ml and 3.8±2.9 pg/ml. The mean value of G-CSF in MM patients was significantly higher than the control group (p<0.01). The serum levels of IL-6 in MM patients was 6.7±13.2 pg/ml, while in the control group it was under the minimal detectable level. The serum TNF-α levels of the patients with MM were 3.9±6.8 pg/ml vs 0.02±0.18 pg/ml of the control subjects, showing a significant higher mean level in the MM patients vs the normal subjects (p<0.05). The correlation coefficients between the LAP score and the serum levels of G-CSF, IL-6 and TNF-α were 0.450 (p<0.001), 0.270 (p<0.05) and 0.380 (p<0.01). Conclusion: The LAP score and the concentration of TNF-α and IL-6 were significantly higher in MM patients vs normal subjects. The most significant correlation was noted between the LAP scores and the G-CSF level. This finding suggests that the increase of the LAP score in MM may reflect a stimulation of the neutrophils by G-CSF.

**1223**

**PROGNOSTIC FACTORS AFTER FIRST COURSE VAD (VINCRISTINE, DOXORUBICIN, DEXAMETHASONE) IN MULTIPLE MYELOMA PATIENTS TREATING FOLLOWING ASCT**

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**Backgrounds.** High-dose melphalan with peripheral blood stem cell rescue represents today the standard therapy for young multiple myeloma (MM) patients. The most frequent therapy inducing remission is chemotherapy according to VAD protocol. Aims. The aim of this study was to determine prognostic factors and overall survival (OS) according to results of first VAD chemotherapy. Materials and methods: The study group consisted of 64 MM patients (32M/32F), median age 57.5 y; range 35-75 yr. Diagnosis was established on the base of common rules. Patients were in the stage of the clinical progression of the disease on the basis of the evaluation of the plasma cell infiltration in bone marrow and monoclonal protein IgG class was observed in the serum at 36 cases of the patients, IgA-11, IgD-1, Benes Jones-15 and nonsecretory MM-1. Patients achieved following types of chemotherapy: 64 pts received chemotherapy according to VAD with following auto-PBSCT. We divided pts in 2 groups: first group: Pts, who are died; OS <740 days (median OS-740 days), second group: Pts, who are living; OS >740 days. We compared results of investigations/studies, which are made to recognise MM before and after first VAD course. All of the results have been statistically tested by using T-test student for the independent groups. For statistically significant results were p<0.05. Results. The baseline serum concentration of creatinine was statistically significantly higher (p<0.05) larger in first group (mean: 4.6 mg/dl ±4.17; range: 0.59-14.36 mg/dl) according to the second one (mean: 1.01 mg/dl ±2.34; range: 0.66-11.3 mg/dl). We detected statistically significant results (p<0.05): - decrease in baseline concentration of monoclonal protein (mean): in first group from 5.5 g/dl (range: 0.56-13.85 g/dl ±3.8) to 4.4 g/dl (range: 0.6-10.5 g/dl ±3.1); in
second one (mean) from 4.7 g/dL (range: 0.6-9.3 g/dL +2.9) to 3.0 g/L (range: 0.6-9.1 g/dL +2.2); reduction of β2-microglobulin in the second group from mean: 8 mg/L (range: 0.46-7.7 mg/L +13.65) to 6 mg/L (range: 0.98-6.7 mg/L +13.28); differences in: baseline 24 hour urine calcium between groups; mean: 3.2 mmol (range: 0.16-15.8 mmol +8.3)-in first group according to: 6.5 mmol (range: 0.14-13.5 mmol +7.8); value of decrease of 24 hour urine calcium between groups: mean: 1.0 mmol (range: 0.19 mmol +0.08)-in first group, mean:0.18 mmol (range: 0.31-3.33 mmol +8.6)-in second one; an increase in 24 hour urine protein in first group (mean): from 2.7 g/l (range: 0.03-8.2 +2.44) to 4.32 g/l (range: 0.15-20.14 +6.8) and a decrease (mean)-in second group: from 1.3g (range: 0-9.2 +2.2) to 0.25g (range: 0.14- +0.3); difference in 24 hour urine protein after first VAD chemotherapy between first and second group: 0.5 g/l (range: 0-32 g/l +20.14; +6.8) and a decrease (mean)-in second group: 0.02g (range: 0-10.8 g/l +2.2). The AUC for ibandronate was not significantly differ-
p<0.02). The AUC for ibandronate was not significantly differ-
rected to placebo. Aims. In this open-label study we assessed the pharmacokinetics and safety of intravenous iban-
dromate, ibandronate, is indicated for use in patients with bone metas-
isms of action), reflected in rises in AP. 2. In contradiction with these findings, we did not find any correlation with the type of response.
Conclusions. 1. Velcade probably has an osteoblastic activity (besides other mechanisms of action), reflected in rises in AP. 2. In contradiction with a recent publication of Žagar et al., there is no correlation between Velcade response and level of AP increase.

1225
RISSE IN SERUM ALKALINE PHOSPHATASE (AP) ARE NOT CORRELATED WITH RESPONSE TO VELCADE (BORTZOEMI)
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Backgrounds. Velandes, a proteasome inhibitor, is a novel agent in the treatment of multiple myeloma (MM), showing promising activity even in relapsed/refractory MM. Recently Zangari and colleagues repeatedly 
effectively claimed a clear correlation between rise in serum AP and activity of Velandes (abstract Sydney '05, oral presentation ASH '05 and Brit J of Haematol '05). Serum AP could be a cheap and easy test to decide on an expensive treatment. We have/had a different impression and studied our myeloma population treated with Velandes, partly in a retrospective, partly in a prospective way. Methods. Between March '03 and August '05 52 evaluable patients were treated with Velandes for relapsed/refractory MM at our institution. 4 Patients presented with a light chain λ, 3 with light chain κ, 5 with IgA κ, 2 with IgAκ, 12 with Igακ, 7 with Igλκ, and finally 1 with a non secreting MM. The youngest patient was 55 years, the oldest 85; 19 were male, 13 female. Prior to Velandes, they were treated 

1226
A SINGLE FIXED DOSE OF PEG-FILGRASTIM ALLOWS ADEQUATE STEM CELL MOBILISATION IN MULTIPLE MYELOMA PATIENTS
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Backgrounds. Autologous transplant is the standard of care for multiple myeloma (MM) patients aged less than 65 years. An adequate mobili-
sation of stem cells is therefore essential to complete the therapeutic program. PEG-filgrastim (PEGF), a long lasting conjugated form of filgras-
tim used as a single dose of 6 mg to shorten chemotherapy induced neu-
ropenia, has been recently evaluated for stem cells mobilization in haematologic malignancies. Preliminary reports show that PEGF is as effective as filgrastim in mobilizing MM patients with a better compli-
ce. Aims. To evaluate the mobilization capacity and the safety of PEGF after DCEP regimen in MM patients enrolled in a high dose program. Methods. We mobilized 11 previously untreated MM patients with a combination of DCEP chemotherapy (Decadron 40 mg/day i.v. days 1-
4, Cyclophosphamide 700 mg/m²/day i.v. days 1-2, Etoposide 100 mg/scm/day i.v. days 1-2, Cis-Platin 25 mg/m²/day i.v. days 1-2) followed by a single subcutaneous dose of PEGF 6 mg 48 hours after the end of chemotherapy. The first leukapheresis was performed when peripheral CD34+ cells were >20/µl and continued until at least 4×10⁹/kg CD34 cells were collected. Patients collecting <2×10⁹/kg CD34 cells were considered poor mobilisers. Results. The median number of CD34+ cells collected with 1 (6 patients) or 2 leukaphereses (5 patients) was 5.9×10⁹/kg (range: 1.5-29.4). Nine patients mobilized 10 days after the end of DCEP therapy, 1 patient after 9 days, 1 patient after 11 days. One patient who had failed the first mobilization with filgrastim, with PEGF mobilization was still unable to mobilize. One patient did not mobilize (1/11: 9%). Median peak number of peripheral CD34+ cells at the time of collection was 60/mL (range: 24-418). Five patients showed WHO grade 3-4 therapy-related neutropenia and 2 WHO grade 2 throm-

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Bocytopenia. No patient experienced fever or infections or required transfusions. The majority of the patients complained of mild to moderate back pain easily controlled by oral analgesics. Conclusions. This study shows that a single fixed dose (6 mg s.c.) of PEGf is safe and effective to mobilize adequate number of CD34+ cells in the majority of myeloma patients, a category usually considered worse mobilizer than patients with other hematological malignancies. In addition, the single administration of PEGf shows better compliance than repetitive doses of filgrastim.

1227 EXANTHEMA AND HERPES ZOSTER INFECTION DURING VELCADE USE INCIDENCE, TREATMENT AND PROFYXILIS
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Backgrounds. Bortezomib has been shown to be highly effective in the treatment of relapsed multiple myeloma (MM). Data from clinical trials show that the incidence of herpetic zoster during bortezomib therapy is about 15%. Skin rash is quite common toxicity seen in MM patients treated with bortezomib. Where reported, its incidence in clinical trials ranged from 8 to 18%. We reported our results and treatment of this two patients who already had VZV reactivation before bortezomib treatment. OS, TRM and SAE of elderly patients (65+) were improved compared to previous studies. These differences could be explained by the population of fit elderly patients with few adverse prognosis factors.

1229 PLASMA CELL IMMUNOPHENOTYPE CD56 POSITIVE AS GOOD PROGNOSIS MARKER
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Background. Malignant plasma cell could have a classical appearance, or it could be atypical at the optical microscopy analysis. This is known to be a B-cell without expression of lineage markers (CD19, CD20), with lack of CD45 expression and typical expression of CD38, CD138, and CD56. There were described aberrant coexpressions of CD10, CD28, c-Ki-ras and in a few cases. Aims. We have analyzed our cases of multiple myeloma and plasma cell leukemia to correlate with the immunophenotype and microscopic appearance. Methods. We performed optical microscopy and immunophenotyping on peripheral blood cells and bone marrow aspirate, in 67 patients, with median age of 65 years. Results. We found atypical microcytic peripheral blood cell in 65.3%, 15.7% of patients with plasma cell leukemia and plasmablasts in 11.9%. Plasmablasts. Immunophenotyping by flowcytometry found in 70% of patients the expression of CD38, CD138 and CD56. In 15% we found lack for CD56 and in 20% lack for CD38. We found also that those patients with lack for CD56 had poor prognosis and the lack for CD38 didn’t change the prognosis. In a patient with plasma cell leukemia positive expression of CD56 could be considered as good prognosis marker, despite of aberrant lack of CD38 expression on plasmablasts. Aberrant coexpression of CD20, CD13 or CD38 didn’t associate poor outcome. In 5% patients we found plasma cells in peripheral blood associated with poor prognosis and terminal phase of disease. In conclusion, our study shows that immunophenotyping in plasma cell leukemia and multiple myeloma is very important, and critical for the quickly diagnosis, too. We can find important prognostic markers, and we consider that lack of expression of CD56 could be the most important, associated with poor prognosis.
Heparin is a potent anticoagulant used in acute venous or arterial thromboembolism. Unfortunately, individual differences can be seen in anticoagulant response of patients that are treated with heparin. Activated partial thromboplastin time (aPTT) is the most widely used monitoring test. The therapeutic range for aPTT can be different such as 1.5-2, 1.5-2, 1.5-3 times of the normal laboratory mean in the literature. aPTT is not correlated with observed heparin concentration or its antithrombotic effect. Different aPTT reagents may have different responses to heparin. This may be the cause of the difference between the therapeutic ranges suggested in the literature. When we consider all of these data, making therapeutic range calibration for each aPTT reagent and corresponding to heparin levels 0.2 - 0.4 U/ml by protamine sulphate titration or anti-Xa level of 0.3 - 0.7 U/ml may be an appropriate approach. Using anti-Xa assay is cheaper and easier than protamine sulphate titration. The aim of this study is to determine the therapeutic aPTT range by using anti-Xa assay and whether there is a difference between these old and new ranges. Besides, a poll is applied among doctors working in different wards of the hospital to understand that these two therapeutic ranges are different from the daily practice. aPTT (STA CK Prest 5; Diagnostica Stago, France) and anti-Xa (STA-Rotachrom_ Heparin; Diagnostica Stago, Fransa) are studied in plasma samples of patients receiving heparin hospitalised in Internal Medicine and Neurology wards because of venous thromboembolism (VTE) or serebrovascular accident (SVA) between September 2002 and June 2003. The correlation between aPTT and anti-Xa was analyzed by two vari- ant correlation analysis and linear regression analysis. There was a very good correlation (r=0.73, p<0.001). The formulation of the correlation was as follow: aPTT= 37 + (68.8xAntiXa). The therapeutic aPTT range calculated using 0.3 and 0.7 U/ml anti-Xa levels were 58-85 seconds. 1.5 - 2.5 times of these values were corresponding to 47.4 - 79 seconds. When a poll was made among 22 doctors treating VTE or SVA, it was seen that the therapeutic ranges used were different individually and from the values found in the study.

1232
DETERMINATION OF THE RELATIONSHIP BETWEEN PLASMA HEPARIN LEVEL AND APTT AND ASCERTAINMENT OF THE APTT RANGE TO BE AIMED DURING TREATMENT OF VENOUS THROMBOEMBOLISM BY HEPARIN
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Heparin is a potent anticoagulant used in acute venous or arterial thromboembolism. Unfortunately, individual differences can be seen in anticoagulant response of patients that are treated with heparin. Activated partial thromboplastin time (aPTT) is the most widely used monitoring test. The therapeutic range for aPTT can be different such as 1.5-2, 1.5-2, 1.5-3 times of the normal laboratory mean in the literature. aPTT is not correlated with observed heparin concentration or its antithrombotic effect. Different aPTT reagents may have different responses to heparin. This may be the cause of the difference between the therapeutic ranges suggested in the literature. When we consider all of these data, making therapeutic range calibration for each aPTT reagent and corresponding to heparin levels 0.2 - 0.4 U/ml by protamine sulphate titration or anti-Xa level of 0.3 - 0.7 U/ml may be an appropriate approach. Using anti-Xa assay is cheaper and easier than protamine sulphate titration. The aim of this study is to determine the therapeutic aPTT range by using anti-Xa assay and whether there is a difference between these old and new ranges. Besides, a poll is applied among doctors working in different wards of the hospital to understand that these two therapeutic ranges are different from the daily practice. aPTT (STA CK Prest 5; Diagnostica Stago, France) and anti-Xa (STA-Rotachrom_ Heparin; Diagnostica Stago, Fransa) are studied in plasma samples of patients receiving heparin hospitalised in Internal Medicine and Neurology wards because of venous thromboembolism (VTE) or serebrovascular accident (SVA) between September 2002 and June 2003. The correlation between aPTT and anti-Xa was analyzed by two vari- ant correlation analysis and linear regression analysis. There was a very good correlation (r=0.73, p<0.001). The formulation of the correlation was as follow: aPTT= 37 + (68.8xAntiXa). The therapeutic aPTT range calculated using 0.3 and 0.7 U/ml anti-Xa levels were 58-85 seconds. 1.5 - 2.5 times of these values were corresponding to 47.4 - 79 seconds. When a poll was made among 22 doctors treating VTE or SVA, it was seen that the therapeutic ranges used were different individually and from the values found in the study.

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PLATELET AGGREGATION ABNORMALITIES IN PATIENTS WITH THROMBOSIS OR RECURRENT FETAL LOSS (A PRELIMINARY REPORT)
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Background. The results of recent studies suggest that platelets have a major role in arterial and venous thrombosis and recurrent fetal loss (RFL). Aims. The purpose of this study is to evaluate the platelet aggrega- tion abnormalities in patients with arterial thromboembolic disorders (ATE), venous thromboembolism (VTE) and RFL. Methods. Results of three ATE (three ischemic stroke), 17 VTE (12 deep vein thrombosis/pulmonary embolism, two portal vein thrombosis, one hepatic vein thrombosis, one renal vein thrombosis and one retinal vein thrombosis) and 28 patients with RFL were compared with 59 controls in this prelimi- nary report. Platelet aggregation was induced by adenosin diphosphate (5 µM) (ADP), collagen (0.2 mg/ml), and epinephrine (10 µM). The analy- ses were performed by using a Whole Blood Lumi-Aggregometer. Cases with ATE were not evaluated as a distinct group in statistical compar- ison because of inadequate case number. Results. The whole patient group (5/48 vs. 0.59, p=0.016) and the group of patients with VTE (2/17 vs. 0.59, p=0.048) have significantly higher ratio of patients with augmented response to ADP than the control group. The whole patient group (8/48 vs. 1.9, p=0.007) and the group of patients with RFL (4/28 vs. 1.59, p=0.056) have a significantly higher ratio of patients with low

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HELICOBACTER PYLORI ERADICATION IN PATIENTS WITH IDIOPATHIC THROMBOCYTONEPURIC PUPPURA
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Idiopathic thrombocytopenic purpura (ITP) is an autoimmune disease which autoantibodies are responsible for the mechanism of platelet destruction. Some studies have reported the presence of Helicobacter pylori infection with autoimmune disease particularly with ITP. There are increasing findings with the association between eradication of H. pylori infection and ITP. The aim of our study to asses platelet count increase after H. pylori eradication therapy in ITP patients. Methods. In the present study we describe 7 ITP patients which H. pylori was diagnosed. This prospective study was carried out in the department of hematology of Gaziantep University between February 2004 and February 2006. There were 1 male, 6 female with a median age of 29 (range 20-48). Mean platelet count was 49,000/µL. Patients who were known to have any chronic or systemic disease, consi- dered at risk for bleeding or platelet count lower than 10,000/µL and those younger than 16 years of age were excluded. Diagnosis of ITP was established if patient had platelet count <100,000/µL for 6 months, exclu- sion of the other causes of thrombocytopenia and normal or megakary- ocytic hyperplasia of bone marrow aspiration. H. pylori infection was assessed by means of a 14C-urea breath test and positivity was defined as positive result on urea breath test. All patients received the anti-H. pylori infection eradication triple therapy (amoxicilin 750mg b.i.d, clar- itromycin 500mg b.i.d, lanproprazole 30 mg b.i.d). Platelet number was monitored every two weeks until six months after H. pylori eradica- tion therapy. In 7 patients 14C-urea breath test were positive. Megakaryocytic hyperplasia was seen in bone marrow 4 of these patients. Results. Three of seven patients platelet count increased at least 30,000/µL of baseline value (median 60000/µL)/µL with H. pylori eradication ther- apy (%42). Four was accepted as non-responder of H. pylori eradication therapy. In the patient of them had been started another treatment and the other two patients platelet counts are 65,000/µL and 54,000/µL respectively. Conclusions. Eradication therapy of H. pylori infection is effective in ITP treatment. Even though the pathogenetic mechanisms of H. pylori dependent thrombocytopenia remain obscure, when H. pylori was estab- lished eradication therapy should be started in ITP patients. But further studies on large number of patients are needed.
response to ADP than the control group. The patients with VTE (8/17 vs. 1/59, p=0.055) have a significantly higher ratio of patients with low response to collagen than the control group. The whole patient group (12/46 vs. 2/59, p=0.001), the group of patients with VTE (5/17 vs. 2/59, p=0.005), and the group of patients with RFL (5/28 vs. 2/59, p=0.083) have a significantly higher ratio of patients with low response to epinephrine than the control group. Sticky platelet syndrome-like abnormality was detected in four cases (8.3%) of the patient group (one case with type I-like in RFL group, one case with type III-like in ATE group, two cases with type III-like in VTE group). Conclusions. Although we need data which will be obtained from many more cases for a certain evaluation, we suggest that in approaching the patients with thrombosis/RFL, platelet functions screening should be applied according the results of this preliminary report.

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PULMONARY EMBOLISM: EPIDEMIOLOGICAL CHARACTERISTICS ACQUIRED AND INHERENT RISK FACTORS
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Backgrounds. Pulmonary embolism (PE) is the most serious form of venous thromboembolism. Aims. To analyze the epidemiological characteristics and search for risk factors among cases of PE occurred in our area (North-Western Greece). Materials and Methods. The study group consists from 125 adult patients (mean age= 62, SD =14.8 years) presented in the emergencies with high PE probability. All patients treated in the Pneumonology Clinic during the last four years. Four patients with definite diagnosis of severe PE, with unstable cardiovascular function that treated on the Intensive Care Unit of our Hospital, are not included. The diagnosis of PE confirmed on 74 patients (74/123, 60%) according to the co-existence of cancer, lymphoma, anti-phospholipid syndrome, recent trauma or operation and/OR prolonged immobilization. Among the 49 patients that we did not confirm a diagnosis of PE (non-PE) only three were found with a condition that could accompany PE. During the follow-up one death occurred that attributed to secondary PE and two patients developed recurrence. Results. Although the women and the older presented more frequently as cases of PE neither the sex nor the age was statistically different between the cases of PE and non-PE patients. Fibrinogen and D-Dimers levels are significantly higher in the non-PE patients. Prothrombin, Factor V, Factor II polymorphism, PC, PS and ATIII activity, fibrinogen and LDH activities are similar between the PE and non-PE patients. Lupus anticoagulant occurs less frequently among the PE than PE cases but this difference was not significant. Common thrombophilia mutations were always found in higher frequency among PE cases than cases of SPE. Heterozygosity for factor V Leiden (6/29, 20.7% on PE versus 0% on SPE cases) heterozygosity for prothrombin G20210A (7/29, 24.1% on PE versus 2/35, 5.7% on SPE cases) and homozygosity for C677T of MTHFR (7/29, 24.1% on PE versus 4/35, 11.4% on SPE cases). Among the patients with PE the heterozygosity for prothrombin G20210A mutation is very common in comparison to normal healthy controls (O.R.= 11.6 95% C.I.=3.8 to 35.0). In a subgroup of PE patients (24/74) mutation is very common in comparison to normal healthy controls (O.R.= 10.7 95% C.I.=2.7 to 42.0). In non-PE patients (48/74) mutation was not significant in comparison to normal healthy controls (O.R.= 1.9 95% C.I.=0.6 to 6.2). Within cancer population the highest risk of VTE is recorded in patients with myeloprolipherative disorders (7/35, 20.5%), 15 patients had concomitant diseases: cardiac (8), renal (1), solid cancer (1), metabolic disease (1), lymphoma, myelodysplasia (1), hematological disease (1), 30 Multiple Myeloma (MM)- entered in this study. Patients with haematological malignancies, b) the incidence of a thrombotic event was increased in cancer patients. The aim of this retrospective study was evaluate: a) the overall incidence of VTE in our patients with haematological malignancies, b) the incidence of a thrombotic status in these patients. Thrombophilic screening consists of Factor V Leiden and Factor II polymorphism, PC, PS and ATIII activity, and LAC and ACA Ig M and Ig G assays. From January 2004 to December 2005, 259 consecutive patients with Acute Leukemias (AL), 34 Hodgkin Disease (HD), 60 Non Hodgkin's Lymphomas (NHL), 64 Myelodysplasia (MDS), 30 Multiple Myeloma (MM) entered in this study. Patients with myeloprolipherative disorders were not included since in these cases it is well known VTE represents a frequent feature of disease. 3. Results. Of the 259 patients, 20 (7.7%) -8 males, 12 females, median age 64 y, (range 20-90 y)- developed a VTE: 11 deep vein thrombosis, 3 inferior vena cava, 6 upper right arm. VTE occurs in 6 (8.4%) AL, 4 (11.2%) HD, 7 (12%) NHL, 1 (1.5%) MDS, 2 (6.6%) MM. 15 patients had concomitant diseases: cardiac (8), renal (1), solid cancer (1), metabolic disorders (5); furthermore 6 of these had central venous catheter, 5 were cancer patients treated > 35days, 5 had haematological disease status at time of VTE; 15 cases had active disease (5 at onset, 11 in relapse or progression) while 5 were in complete remission (CR). Thrombophilic screening was available in 16 patients. Abnormal
tests were detected in only 4 (25%) cases; ACA IgG high title (1), reduced PC activity (1), hyperhomocysteinemia (2). In all cases VTE treatment has been successfully done with subcutaneously LMWH for 4 months at least. To date 18 of 20 patients are alive: 11 in hematological CR, 7 in stable disease, 2 patients died because of progressive disease. 4. Conclusions. In our series, if small, 75% of patients who developed VTE had an active phase of hematological disease, VTE incidence was higher in lymphoproliferative disorders with respect to other malignancies. with high abnormal thrombophilic tests were detected in only 25% of cases. The endovascular wall damage induced by central venous catheter plus chemotherapy proved to be determinant in the development of upper right arm VTE.

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IS OSTEONECROSIS ASSOCIATED WITH MUTATIONS OF THE METHYLMETHYTHREDOFOLATE REDUCTASE GENE?
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Backgrounds. Mutations of the methylenetetrahydrofolate reductase (MTHFR) gene interfere with homocysteine metabolism, and hyperhomocysteinemia is considered a risk factor for thromboembolic complications. Intravascular coagulation represents the major pathogenic pathway leading to ischemic bone death (osteonecrosis) and the role of throbophilic gene mutations is increasingly being recognized. Aims. The purpose of our study is to investigate the presence of MTHFR mutations in patients with osteonecrosis (ON) in an effort to clarify the complex pathogenesis of this disease. Methods. We evaluated a patient group of 48 consecutive adults with ON and a control group of 48 healthy blood donors. All controls were matched for race, age, and gender to the patients and had no history of cardiovascular disease or thromboembolic events. Genetic analysis of the MTHFR C677T and A1298C polymorphisms was carried out by allele-specific polymerase chain reaction. Results. Homozygosity for the MTHFR C677T mutation was present in 63% (3/48) of ON patients compared to 8.3% (4/48) of controls. The difference was not statistically significant, with an odds ratio of 0.7 (95% confidence interval 0.2 to 3.5). Homozygosity for the MTHFR A1298C mutation was present in 12.5% (6/48) of ON patients compared to 10.4% (5/48) of controls. The difference was again not statistically significant, with an odds ratio of 1.2 (95% confidence interval 0.3 to 4.3). Conclusions. Although hyperhomocysteinemia is considered a thrombophilic factor, the potential pathogenic role of the C677T and A1298C MTHFR mutations in thromboembolic disease remains controversial. In the current report, we detected no differences in the prevalence of these mutations in patients with ON compared to controls. Intravascular coagulation in patients with ON may be mediated by other genetically determined factors.

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DERMATAN SULPHATE THERAPY FOR HEPARIN-INDUCED THROMBOCYTOPENIA
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Heparin-induced thrombocytopenia (HIT) is an acquired hypercoagulable state. As soon as HIT is suspected, heparin should be discontinued and non-heparin anticoagulant should be started. Lepirudin, Argatroban and Danaparoid are among the alternative anticoagulants more investigated in HIT. Dermatan sulphate (DS) is a safe, effective and inexpensive therapeutic option for HIT though clinical experience with its use is limited. We report our clinical experience on DS therapy in patients with HIT. HIT with thrombotic syndrome was clinically suspected in 3 patients according with the Warkeinten’s criteria (NEJM 2001;344:1256-1292). Laboratory confirmation of HIT was obtained by an ELISA test for anti-PF4-heparin antibodies in two patients. Venous thromboembolism was confirmed by imaging tests. DS (Mediolanum Farmaceutici, Milan, Italy) was administered by intravenous continuous infusion (rate 0.6 mg/Kg/h), the infusion was regulated monitoring APTT every 6-12 hours and targeting APTT of 1.5-2 times the normal value. Platelet count was close monitored. Warfarin was started in two cases when platelet count rised over 100 x 10^9/L, in one case before DS infusion. DS was stopped when INR was in therapeutic range for two consecutive values. The cost by vial of DS is 0.9/75 euro in Italy. Patients included were two females (59 and 81 years old) and one male (65 years old). In one case HIT developed during administration of unfractionated heparin for lower limbs deep venous thrombosis (DVT) and the course was complicated by sinus thrombosis, in two cases after antithrombotic prophylaxis with low-molecular-weight heparin: one, with enterovascular fistula, the other with enterovesical fistula, suffered of upper limbs deep vein catheter-related thrombosis and pulmonary embolism. In 2 patients DS was started with platelet count of 49-129 x 10^9/L and platelet count rised over 150 x 10^9/L after 3-5 days, DS infusion was continued for 11 and 13 days; in patient, taking Warfarin, DS was started with platelet count of 109 x 10^9/L and DS was stopped with platelet count of 62 x 10^9/L. No bleeding complications or adverse events were observed, clinical improvement was observed in all patients. Patient with enterovesical fistula, few weeks later HIT, was operated and antithrombotic prophylaxis with DS offered an uneventful outcome. DS total therapy cost for two patients, before warfarin was in therapeutic range, was respectively of 26.7 euro and 56 euro. DS appears an effective and safe therapeutic option for patients with HIT. Our experience, together with other reports in which patients with HIT were treated successfully, encourages using this alternative anticoagulant drug in HIT therapy and as postoperative antithrombotic prophylaxis in patients with recent history of HIT. DS shows also a favourable profile cost-benefit.

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THROMBOMODULIN, SEPCR AND DI-DIMERS AS PLASMA MARKERS OF ENDOTHELIAL DYSFUNCTION IN WOMEN WITH A HISTORY OF RECURRENT PREGNANCY LOSS
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Venizelion Hospital, HERAKLION, Greece; Hemostasis and Thrombosis Department, Blood Bank Center, Venizelion Hospital, Heraklion Crete, Greece

Recurrent pregnancy loss can be associated with endothelial disturbance (whether activation, dysfunction or damage). Thrombomodulin (TM) and the endothelial protein C receptor (EPCR) are glycoprotein receptors expressed mainly on the endothelial surface of blood vessels and also in the placenta. They both play a key physiological role in the protein C anticoagulant pathway. Defects in these proteins might play an important role in the pathogenesis of fetal loss. So we decided to study Di-Dimers, TM and SEPCR as early markers for the beginning and prognosis of recurrent pregnancy loss. We studied 102 women with unexplained fetal loss and 44 women as control group. We used an immunologic assay (Dade Behring) for calculating Di-Dimers and ELISA (Asserochrom ASTAGO) for TM and SEPCR measurement. The levels of Di-Dimers were 189.28±15.7 µg/L in patients group and 172.02 µg/L in control group (p=0.46). TM levels were 9,78±2,4 ng/ml in patients group and 8,3±2,7 ng/ml in controls (p=0,017). There was not statistical difference in SEPCR measurement (187.2 ng/ml versus 160,7 ng/ml in control p=0,22). TM may serve as a clinically meaningful endothelial injury marker in women with a history of recurrent pregnancy loss. Further investigation is needed to see the significance of other factor as Di-Di and SEPCR.

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THE ABO BLOOD GROUPS, VIII, FIX, VWF LEVELS AND LEIDEN IN PATIENTS WITH THROMBOSIS IN GREECE
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Increased levels of VIII, FIX, VWF in plasma as the presence of FV leiden represent an important risk factor for venous thromboembolic disease. There is also a relationship between these factors and ABO Blood groups. We investigated the influence of the ABO blood group in Greek patients with thrombosis and the association with raised plasma levels of the above coagulation factors. 159 patients of median age 48,5 ± 5,4 (97 females and 62 males) were included in our study. They have visited our hospital for first thrombotic event when they were younger than 60 years old. Patients with malignancies or with history of liver failure or nephrotic syndrome were excluded. As controls 60 (36 F and 24M) apparently healthy individuals were recruited. We measured the plasma levels of
Results. The sample included 41 patients with arterial thrombosis, 99 patients with venous thrombosis, and 125 healthy controls. The average age of the three groups was 42.8±19.4, 39.6±17.5, and 35.4±18.6 years respectively. Table 1 shows the clinical presentation of patients with thrombosis. Patients with venous thrombosis were 7.1 times more likely to have Factor V mutation (heterozygous or homozygous) as compared to the control group. On the other hand, neither prothrombin nor MTHFR mutation was significantly associated with venous thrombosis. A logistic regression was conducted to test whether prothrombin and MTHFR mutations increase the risk of venous thrombosis among subjects with Factor V mutation. None of the two factors was found to be significantly associated with venous thrombosis after controlling for Factor V. Patients with arterial thrombosis were 2.5 and 4.4 times more likely to have Factor V (p=0.04) and prothrombin (p=0.04) mutations as compared to the control group. There was no significant association between MTHFR mutation and arterial thrombosis. Upon controlling for Factor V in the logistic regression, prothrombin mutation was 5.3 times more likely to be present among patients with arterial thrombosis. Conclusions. In Lebanon, the presence of prothrombin and/or MTHFR mutations does not seem to influence the risk of venous thrombosis in Factor V Leiden carriers. However, in patients with arterial thrombosis, the risk is increased in Factor V Leiden carriers with prothrombin mutation. These results might have an influence on the risk assessment and management of patients with arterial thrombosis. However, no final conclusions can be made from our results because of the small sample size. It would also be rational to conduct similar studies with stratification of patients into subgroups based on the definite site of thrombosis.

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THE CONTRIBUTION OF PROTHROMBIN AND MTHFR MUTATIONS TO VENOUS AND ARTERIAL THROMBOEMBOLISM IN CARRIERS OF FACTOR V LEIDEN
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Backgrounds. Given the multifactorial aspect of thrombophilia, the identification of combined genetic factors in patients with thrombotic episodes is important to a more accurate risk assessment. These patients are routinely screened for Factor V Leiden (G1691A), prothrombin G20210A, and MTHFR C677T. The population in our study consisted of a group of patients presenting with thrombosis over a period of 18 months. Patients were screened for the three most common thrombophilia genetic mutations namely Factor V Leiden mutation (G1691A), prothrombin G20210A and MTHFR C677T. A group of healthy controls was also included in the analysis. The DNA of patients and controls was extracted using the PEL-FREEZE extraction kit (PEL-FREEZE, DYNAL, USA) and stored at -80°C for later use. Simultaneous testing for all three mutations was done using the Reverse Hybridization StripAssay (Vienna Lab). Extraction, PCRamplification, and Hybridization steps were all followed upon the recommendation of the manufacturer.

Table 1. Clinical presentation of patients with thrombosis.

<table>
<thead>
<tr>
<th>Arterial thrombosis</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebrovascular accident</td>
<td>25</td>
</tr>
<tr>
<td>Peripheral vascular disease</td>
<td>9</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Venous thrombosis</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deep vein thrombosis</td>
<td>47</td>
</tr>
<tr>
<td>Superficial thrombophlebitis</td>
<td>13</td>
</tr>
<tr>
<td>Pulmonary embolism</td>
<td>12</td>
</tr>
<tr>
<td>Portal vein thrombosis</td>
<td>8</td>
</tr>
<tr>
<td>Sagittal sinus thrombosis</td>
<td>5</td>
</tr>
<tr>
<td>Transverse sinus thrombosis</td>
<td>4</td>
</tr>
<tr>
<td>Subclavian vein thrombosis</td>
<td>4</td>
</tr>
<tr>
<td>Central retinal thrombosis</td>
<td>3</td>
</tr>
<tr>
<td>Mesenteric vein thrombosis</td>
<td>3</td>
</tr>
</tbody>
</table>

Results. The prevalence of the heterozygote and homozygous variants for Factor V G1691A (FVL-Leiden, FVL), Factor II G20210A (PT) and methylene-tetrahydrofolate reductase C677T (MTHFR) polymorphisms in the incidence of deep venous thrombosis (DVT). Patients and Methods. We enrolled 128 patients with first episode of DVT (65 males, 63 females) and 186 healthy individuals (83 males, 103 females). FV, FI and MTHFR genotypes were analyzed using PCR amplification. We calculated odds ratio (OR) with 95% confidence intervals (CI), adjusted for gender and age by means of multiple logistic regression. Results. The prevalence of the heterozygote and homozygous variants for FVL (25.0% vs 6.5%, p<0.001) and PT (10.2% vs 3.2%, p=0.011) were higher among DVT patients compared with controls. However, the presence of the T/T genotype for MTHFR was not different between the two groups (9.4% in patients vs 8.1% in control group, p=0.684). In order to evaluate independent and combined effect of the above mutations on the incidence of DVT, we divided the entire cohort into seven groups according to the presence of none, one or two mutations. The combination of the three mutations was not detected. The group without any mutation was used as reference group. Both FVL and PT significantly increased the risk for DVT compared to the reference group (FVL: OR=4.0, 95%

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ARE SEMINAL FACTORS IX AND IXA INVOLVED IN THE SEMINAL COAGULUM FORMATION?
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Backgrounds. In spite of evidence demonstrating the importance of the seminal coagulation and liquefaction process in terms of global fertility and that the seminal coagulum is composed of fibrin-like material, it has rarely been studied from the conventional haemostatic factors perspective. Aim: To investigate Factor (F) FIX and FIXa in human semen. Materials and Methods. Using a one stage factor assay based on PT/APTT and spectrozyme FXa assay FIX and FIXa were studied in a total of 119 semen specimens obtained from sub-fertile, normally fertile, fertile sperm donors and vasectomy subjects. Results. Both FIX and FIXa were quantifiable in human semen. There was a wide individual variation in FIX and FIXa levels within groups. Despite the group size, statistically significant associations with fertility-related parameters were infrequent. There was also a positive correlation between FIX and its activation product, FIXa (n=36; r=0.51; p=0.05). Factor IXa elevation in the high sperm-clump group was significant (p<0.05) and days of abstinence correlated with FIXa levels (n=63; r=0.3; p<0.05). Conclusion: The key finding of this study is that both FIX and FIXa are present in concentrations not dissimilar to plasma levels and apparently functional, as the activated form is also present.

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PREVALENCE OF FACTOR V G1691A, FACTOR II G20120A AND MTHFR C677T POLYMORPHISMS IN PATIENTS WITH DEEP VENOUS THROMBOSIS
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Backgrounds. The risk of thrombosis may occur through the interaction of both genetic and acquired factors. The predisposition towards thrombosis increases with the number of risk factors present in the patient. Aim: The aim of this study was to evaluate the independent and combined effect of Factor V G1691A (FV-Leiden, FVL), Factor II G20210A (PT) and methylene-tetrahydrofolate reductase C677T (MTHFR) polymorphisms on the incidence of deep venous thrombosis (DVT). Patients and Methods. We enrolled 128 patients with first episode of DVT (65 males, 63 females) and 186 healthy individuals (83 males, 103 females). FV, FI and MTHFR genotypes were analyzed using PCR amplification. We calculated odds ratio (OR) with 95% confidence intervals (CI), adjusted for gender and age by means of multiple logistic regression. Results. The prevalence of the heterozygote and homozygous variants for FVL (25.0% vs 6.5%, p<0.001) and PT (10.2% vs 3.2%, p=0.011) were higher among DVT patients compared with controls. However, the presence of the T/T genotype for MTHFR was not different between the two groups (9.4% in patients vs 8.1% in control group, p=0.684). In order to evaluate independent and combined effect of the above mutations on the incidence of DVT, we divided the entire cohort into seven groups according to the presence of none, one or two mutations. The combination of the three mutations was not detected. The group without any mutation was used as reference group. Both FVL and PT significantly increased the risk for DVT compared to the reference group (FVL: OR=4.0, 95%
pared to FVL or PTH only.

Increased the odds of development of DVT.

PTH, but not MTHFR, were important independent risk factors for DVT. In addition, the combination of FVL with PTH or MTHFR further increased the odds of development of DVT.

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GELATINOUS BONE MARROW TRANSFORMATION IN A PATIENT WITH LEUKOPENIA AND ANOREXIA: CASE REPORT AND REVIEW OF THE LITERATURE

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Backgrounds. Gelatinous bone marrow transformation (GMT) is a rare disorder of unknown pathogenesis, characterized by fat cell atrophy, focal loss of hematopoietic cells, and deposition of extracellular gelatinous substances histochemically the amorphous background consists of mucopolysaccharides, rich in hyaluronic acid. The spectrum of underlying diseases are heterogeneous and age-dependent but GMT is commonly associated with weight loss and cachexia. It is still unclear whether the gelatinous transformation is a primary or secondary change but it may be a reversible lesion if the underlying disorder can be eliminated and an adequate nutritional intake be reestablished. Patient/case report. We are presenting a case of gelatinous transformation of the bone marrow in a 40-year-old female who presented with cachexia, night sweats and leukopenia. The receptionist of a gynaecological outpatient clinic and mother of three children had a history of 5 kg weight loss within the last 2 years. Furthermore she reported about amenorrhea since 5 years, hypothyroidism and lack of zinc. Alcoholism or nicotine within the last 2 years. Furthermore she reported about amenorrhea which was caused by excessive consumption of lemon juice. The thyroid was without pathological findings. Laboratory data showed leukopenia (white blood cells, 2.6/μl, hemoglobin 14 g/dl, hematocrit 38.4%, platelets 160/μl, neutrophils 52%, lymphocytes 42%, monocytes 4%, eosinophils 2%). Liver function tests were abnormal: Serum aspartate aminotransferase (AST) 53 U/l (<51U/l), alanine aminotransferase (ALT) 56 U/l (<54 U/l), lactate dehydrogenase (LDH) 521 U/l (<240 U/l), Vitamin B12 (1292 mg/l; normal 200-1100) and folate (16.5 mg/l; normal 2,5-17) were elevated. Zinc was decreased (9.7 mmol/l, normal 11-23 mmol/l), TSH and free thyroxine were abnormal: Serum aspartate aminotransferase (AST) 53 U/l (<51U/l), alanine aminotransferase (ALT) 56 U/l (<54 U/l), lactate dehydrogenase (LDH) 521 U/l (<240 U/l), Vitamin B12 (1292 mg/l; normal 200-1100) and folate (16.5 mg/l; normal 2,5-17) were elevated. Zinc was decreased (9.7 mmol/l, normal 11-23 mmol/l), TSH and free thyroxine were normal under substitution. Auto-antibody tests were not elevated. Viral studies, including HIV, hepatitis, CMV and EBV, were negative. The amorphous gelatinous substance was identified as acid mucopolysaccharide on acidic blue staining at pH 2.5. On a bone marrow biopsy granulopoiesis was reduced and in the intratrabecular space eosinophilic extracellular material and fat cell atrophy, consistent with gelatinous bone marrow transformation was found. Summary/Conclusions. GMT is a rare disorder that is associated with various underlying diseases, the most frequent being anorexia nervosa and the acquired immunodeficiency syndrome (AIDS). Although frequently associated with weight loss, the bone marrow changes have not been associated with any specific deficiency state and their pathogenesis has not been fully elucidated. GMT may act as an indicator of severe illness in a patient but is not indicative of a particular disease. It is an uncommon cause of cytopenia and should be considered in the setting of malnutrition.

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WHAT ARE THE DECISION MAKING PROCESSES OF HAEMATOLOGY PATIENTS WHEN ASKED TO PARTICIPATE IN A PHASE III CANCER CLINICAL TRIAL, IN A DISTRICT GENERAL HOSPITAL

N. Singer

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The objective of this research was to explore the decision making processes of patients who have been asked to consider participating in a Phase III Haematology cancer clinical trial. This was done, by undertaking a qualitative study, using semi-structured interviews in a District General Hospital, within the West of Scotland. Seven participants were purposively selected to participate who had been newly diagnosed with a haematological malignancy. An extensive literature review was undertaken prior to the study with the decision made to conduct semi-structured interviews. These were conducted with participants and tape-recorded. Participants were asked about their decision making processes when they were approached to consider taking part in a Phase III Haematology cancer clinical trial. A thematic analysis was conducted using the long-table approach. The following themes emerged from the study: The timing of the request to participate, the effect of altruism, the process of randomisation and the quality of information that is provided to potential participants. All had an impact on the decision making process of patients when considering participating in a Phase III trial. The findings of this study suggest that further research into why patients choose not to participate in Phase III trials is worthy of consideration. Furthermore the implementation of a training intervention programme aimed at improving healthcare professional communication with cancer patients is also recommended.

1246

HEPATITIS B VIRUS REACTIVATION AFTER CHEMOTHERAPY AND IMMUNOTHERAPY IN NON-HODGKINS LYMPHOMA: REPORT OF TWO CASES

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Backgrounds. Reactivation of hepatitis B virus infection in subjects receiving cytotoxic treatment for hematological malignancies occurs in 20%-50% of chronic HBsAg carriers and in an unknown number of HBsAg negative subjects harbouring occult HBV infection. Immunotherapy with monoclonal antibodies against CD epitopes on lymphocytes produces deep immunosuppression. Case presentation: This paper reports two patients suffering from Non-Hodgkin’s Lymphoma (NHL). Patient no.1 was a 62 y/o man, a case of NHL and chronic HBsAg carrier, too. He received four courses of chemotherapy with fludarabine without serious complication but because of poor response and CD20 positivity in more than 80% of lymphocytes, rituximab, an anti-CD20 monoclonal antibody prescribed for him. After the 4th course of therapy, severe hepatitis developed. Viral study revealed increased serum HBV-DNA from 12×10^6 to >10×10^6 copies/mL. Before starting lamivudine, patient died due to hepatic failure and encephalopathy. Patient no.2 was a 47 y/o man with a questionable history of HBsAg positivity about 25 years ago but this test was negative prior to chemotherapy. He received CHOP regimen and rituximab and after the 6th course of therapy severe hepatitis developed. Viral study revealed positive HBsAg and HBV-DNA >100×10^6 copies/mL. Lamivudine 100mg/day started but after one week he died because of massive uncontrollable widespread bleeding. Conclusion: Considering the results of the published data and a high rate of hepatitis B virus reactivation in cancer patients undergoing chemotherapy and immunotherapy, it is necessary to evaluate hepatitis B and C viral markers including at least HBsAg, HBsAb and HBCAb and HCV Ab pri-

Figure 1. Bone marrow aspirate stained with May-Grünwald Giemsa.
or to therapy and also an international protocol for managing patients at risk for reactivation of hepatitis B virus in high prevalent areas such as Iran should be carried out.

1247
A 3 YEAR SURVEY OF STRAINS IDENTIFIED IN BLOOD CULTURES IN A CLINICAL HEMATOLOGY UNIT
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Febrile neutropenic cancer patients are at the risk for development of serious infections, morbidity and mortality. Among these infections, bacteremia had a place of choice and is associated with a strong mortality. The microbiological documentation is not always present, the antibiotic therapy remain probabilist inspired of the ecology of the service. The aim of this study is to analyze the bacteriological profile of bacteremia in a clinical haematology unit in order to give better the antibiotic therapy of first intention. All the microorganisms(n=138) collected over 3 years(January 2003 to December 2005), from blood cultures of hospitalized patients in the clinical haematology unit were studied. Antimicrobial susceptibility testing has been carried out by disk diffusion method as referred to the French Society of Microbiology. All susceptibility data were stored in a laboratory data base using Whonet software. Duplicate isolates defined as the same bacterial species for the same patient with the same profile of susceptibility were excluded. Gram positive cocci rate(GPC) was 60,1% and Gram negative bacilli(GNB) 39,9%. Evolution in time showed an equal rate between GPC and GNB in 2003(51,4% versus 48,6%), then an increase of GPC rate isolated in bacteremia was observed in 2004(63,6%) and 2005(63%).The most frequently identified species were coagulase-negative staphylococci(CNS): 42,7%, Pseudomonas aeruginosa:10,9%, Klebsiella pneumoniae:10,9%, Escherichia coli:7,6%, and Staphylococcus aureus:8,7%. The rate of methillin resistant staphylococci was 25% in S.aureus and 50, 8% in CNS; ciprofloxacin and 85,7% to amikacin. The frequencies of resistance to K. pneumoniae, 86,7% of strains were resistant to ceftazidime, 46,7% to ceftriaxone and 41,7%.Imipeneme and colistin were the most active agents against K. pneumoniae and E. coli (resistance rate= 0%). Bacteremia were respectively 57,1% in 2003 versus 95,5% in 2005.All strains of K. pneumoniae, 50% of strains were resistant to cefazidime, 50% to imipenem, 51, 6% to amikacin, 41,7% to colistin. An increase of the imipenem resistance in P. aeruginosa was observed from 2003 to 2005(28, 6% in 2003 versus 45, 5% in 2005). The incidence of antimicrobial resistance has markedly increased during 2005, especially for the ceftazidime in K. pneumoniae (95, 5% in 2005 versus 57, 1% in 2003) and the imipenem in P. aeruginosa (45, 5% in 2005 versus 28, 6% in 2005). After this study, a restriction of the use of ceftazidime which utilized in the first antibiotic therapy was instaurated in the unit. The ongoing surveillance of antimicrobial resistance in the hematology unit should be helpful in formulation of effective guidelines for therapy.

1249
PROPHYLAXIS OF INVASIVE Fungal INFECTIONS (IFI) IN ACUTE NON LYMPHOID LEUKEMIA (ANLL): Efficacy of AMPHOTERICIN B LIPID COMPLEX (L-AMB) SINGLE LARGE DOSE DURING INDUCTION
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Up to now the optimal prophylactic regimen to prevent IFI in ANLL is not yet been identified. The L-AMB has been used in patients refractory or intolerant to other antifungal drugs, although long time is required to resolve overt infection and therapy failure. We studied 21 ANLL patients treated in 2003(51,4% versus 48,6%), then an increase of GPC rate isolated in bacteremia was observed in 2004(63,6%) and 2005(63%).The most frequently identified species were coagulase-negative staphylococci(CNS): 25% in S.aureus and 50, 8% in CNS; no VISA (vancomycin intermediate S. aureus) was detected during the study period. P. aeruginosa resistance was 33, 4%, 30, 8%, 40% respectively for ceftazidime, imipenem and amikacin. Concerning K. pneumoniae, 86,7% of strains were resistant to ceftazidime, 46,7% to ceftriaxone and 41,7%.Imipeneme and colistin were the most active agents against K. pneumoniae and E. coli (resistance rate= 0%). Bacteremia were mainly caused by coagulase-negative staphylococci during the three years study. Multiresistance of gems isolated is worrying limiting the therapeutic choice. Ongoing cooperation between haematologists and microbiologists is important to detect trends in epidemiology which can be used to design empirical antibiotic regimes and guide infection control policies.

1248
BACTERIAL FLORA AND ANTIBIOTIC RESISTANCE IN A CLINICAL HEMATOLOGY UNIT
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Infections are among the most serious complications in neutropenic patients and are associated with an increased morbidity and mortality. Ongoing surveillance of infection in neutropenic patients is essential to detect changes in epidemiology and to guide better empirical antibiotic regimes and infection control policies. The aim of this study is to analyze the bacterial flora and the antibiotic resistance of isolates in a clinical haematology unit during three years period. From 1 January 2003 to 31 December 2005, 437 strains were isolated from different specimens. Antimicrobial susceptibility testing has been carried out by disk diffusion method as referred to the French Society of Microbiology. All susceptibility data were stored in a laboratory data base using Whonet software. Duplicate isolates defined as the same bacterial species for the same patient with the same profile of susceptibility were excluded. Gram negative bacilli(GNB) rate was 47,1% and Gram positive cocci(GPC) rate 52,9%. The most frequently identified species were Coagulase negative staphylococci(CNS): 29,3%, Escherichia coli:14%, Staphylococcus aureus: 10,7%, Klebsiella pneumoniae: 9,1% and Pseudomonas aeruginosa:7,5%. The global rate of methillin resistant Staphylococci was 27,7% in S. aureus and 61,4% in CNS, no VISA (vancomycin intermediate S. aureus) was detected during the study period. For E. coli, the frequencies of resistance to ceftazidime, ciprofloxacin and amikacin were respectively: 45%, 26,3% and 21,3%. Concerning K. pneumoniae, 84, 8% of strains were resistant to ceftazidime and were producing extended spectrum β-lactamase (BLSE). The evolution in time showed an increase in rate of K. pneumoniae BLSE: 57, 1% in 2003 versus 95, 5% in 2005. All strains of K. pneumoniae isolated remained sensitive to imipenem and colistin. Concerning P. aeruginosa, 50% of strains were resistant to ceftazidime, 50% to imipenem, 51, 6% to amikacin, 41,7% to colistin. An increase of the imipenem resistance in P. aeruginosa was observed from 2003 to 2005(28, 6% in 2003 versus 45, 5% in 2005). The incidence of antimicrobial resistance has markedly increased during 2005, especially for the ceftazidime in K. pneumoniae (95, 5% in 2005 versus 57, 1% in 2003) and the imipenem in P. aeruginosa (45, 5% in 2005 versus 28, 6% in 2005). After this study, a restriction of the use of ceftazidime which utilized in the first antibiotic therapy was instaurated in the unit. The ongoing surveillance of antimicrobial resistance in the hematology unit should be helpful in formulation of effective guidelines for therapy.
CHEMOTHERAPY FOR AML AND/OR ALLOGENEIC TRANSPLANTATION ACCORDING TO EORTC/MSG CRITERIA IN PATIENTS UNDERGOING HIGH DOSE AUDIT OF THE USE OF ANTIFUNGALS AND THE ACTUAL RATES OF FUNGAL INFECTION

men. Antifungal drug use and actual rates of invasive fungal infection in patients with AML and/or allograft transplant recipients admitted to Bartholomew’s Hospital, LONDON, United Kingdom

Voriconazole could be more effective agents.

to amphotericin B, 5-Fluocitosine, and fluconazole. Itraconazole and

cussion

reaction. He did not receive any further antifungal therapy and the skin fever but it was discontinued after one dose because of a severe infusion

On day +9 he was started on liposomal amphotericin B for neutropenic

Metarrhizium anisopliae that the DNA amplified from tissues belonged to the fungal species

plex. Subsequent sequencing of amplified fragments and comparison

normal. Tissue samples were sent to the Mycology Reference Labora-
tures and a galactomannan test were negative. A chest CT scan was

appeared involving the face, trunk, and limbs. A skin biopsy yielded dermoepidermic necrosis and fibrin thrombi. Not cultures were obtained. The patient was receiving antibiotics and caspofungin for persistent neutropenic fever. He became afebrile and recovered from his neutropenia four days later. Caspofungin and antibiotics were withheld. The skin lesions gradually improved. Two more cycles of chemotherapy were administered and two new lesions appeared after the fourth cycle. They gradually resolved. During September and October 2005, while awaiting for an autologous stem cell transplantation (SCT), disseminated skin lesions reappeared. A new skin biopsy was performed and was initially interpreted as an acute inflammatory dermal lesion with a mixed neutrophilic and histiocyte infiltrate. During this time most lesions underwent spontaneous resolution, but new papules appeared. On November 2005 he was admitted to undergo an autolo-
gous SCT. He had then 4 skin lesions in resolution. Deeper sections of the second skin biopsy revealed a nidi of fungi in the dermis with broad hyphae. A new skin biopsy showed similar features. Biopsy cultures on galactomannan test were negative. A chest CT scan was normal. Tissue samples were sent to the Mycology Reference Labora-
tory of Spanish National Center for Microbiology. Specimens were analysed using a panfungal PCR-based assay designed to amplify the internal transcribed spacer regions 1 and 2 from fungal RNA gene complex. Subsequent sequencing of amplified fragments and comparison with sequences of other fungal species included in databases led to know that the DNA amplified from tissues belonged to the fungal species Metarrhizium anisopliae. During transplantation no new lesions appeared. On day +9 he was started on liposomal amphotericin B for neutropenic fever but it was discontinued after one dose because of a severe infusion reaction. He did not receive any further antifungal therapy and the skin lesions were resolved. In December 2005, he showed 2 new skin papules. A biopsy was performed and cultures and PCR for fungal DNA were negative. Voriconazole was started and the lesions disappeared. Treatment was discontinued after a month. No further skin lesions appeared. Dis-
cussion: We report the first case of a probable disseminated infection caused by M. anisopliae in an adult patient. The organism was not iso-
lated and was identified by PCR techniques. This case exemplifies the clinical usefulness of molecular methods to diagnose mycosis due to emerging pathogens. Metarrhizium anisopliae is a com-
motor insect pathogen and occasionally causes infection in animals and humans. To date, there are only 9 reported cases of disease in humans: two of keratitis, two of sinussitis, and one of a disseminated invasive infection in an immunocompromised child. There is no standard treat-
ment. Susceptibility testing suggests that M. anisopliae may be resistant to amphotericin B, 5-Fluocitosine, and fluconazole. Itraconazole and Voriconazole could be more effective agents.

AIDS AND/OR ALLOGENEIC TRANSPLANTATION

Background. The high morbidity and mortality of fungal infections in neutropenic patients has led to prophylactic and empirical drug regi-
mens. Antifungal drug use and actual rates of invasive fungal infection (IFI) may differ considerably. Aims. To audit the use of antifungal drugs in patients with AML and/or allograft transplant recipients admitted to our hospital from 01/01 to 31/12/2004 and to apply the EORTC/MSG criteria (1) for IFI. Methods. The medical notes were retrospectively reviewed for: conversion from prophylactic to empirical treatment; time to neutrophil recovery; duration of hospitalisation and mortality. Primary prophylaxis was with fluconazole 400mg od, while empirical therapy (ambisome) was prescribed for persistent neutropenic fever despite 72-96 hours of antimicrobials. Those patients, who did not meet EORTC/MSG possible criteria, were termed unlikely. Galactomannan testing was not done routinely in our hospital. Results. 54 patients (out of 177 eligible) were assessable, providing 157 episodes. 75% underwent intensive chemotherapy for AML/MDS, 18% allogeneic transplantation and 7% supportive treatment for neutropenic sepsis on the background of AML. 114/157 episodes (75%) received primary prophylaxis - oral flu-
conazole (78%); 21/137 (15%) secondary prophylaxis - oral voricona-
azole. 21/137 (15%) did not receive prophylaxis. The conversion rate from prophylaxis to empirical therapy was 25% (85/337), mainly due to persistent neutropenic fever. These 35 episodes concerned 27 of the 54 patients (50%). The EORTC/MSG infection rates are shown in Table 1.

Table 1.

<table>
<thead>
<tr>
<th>EORTC-defined infection</th>
<th>Number of episodes that were started on empirical treatment</th>
</tr>
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<tbody>
<tr>
<td>Proven</td>
<td>0/35 (0%)</td>
</tr>
<tr>
<td>Probable</td>
<td>4/35 (11%)</td>
</tr>
<tr>
<td>Possible</td>
<td>18/35 (51%)</td>
</tr>
</tbody>
</table>

Of these 35 episodes only 4 (11%) were probable IFI - all had HRCT evidence of IFI with negative bacterial cultures. Another 22 episodes involved HRCTs: 11 had normal results, and 11 had non-specific changes. Thus, HRCT was the main diagnostic test in the small total number of probable infections. Patients in 25 of the 35 episodes on treat-
ment (71%) were hospitalised for >28 days (range 29-60, median 34). Their EORTC/MSG IFI score was: 13 possible, 5 probable, 6 unlikely and 3 not documented. In 3/35 episodes (9%) patients on treatment died during admission - none were related to IFI (2 died of AML; 1 of non-fun-
gal pneumonia); all had possible IFI. Time to neutrophil recovery (> 0.5 x 10^9/L) in 20/35 episodes on treatment (57%) was ≥ 22 days (range 22-
41, median 28): 10 had possible IFI, 2 probable, 6 unlikely and 2 were not documented. Summary/Conclusions. The EORTC/MSG IFI rate - possi-
ble/probable/proven - was only 22/137 (16%) episodes. This may be due to effective prophylaxis and/or early initiation of empirical treatment, but it also reflects the fact that the EORTC/MSG criteria were not intended for routine clinical use. Our audit data aims to allow regular review of anti-fungal policies, but is limited by its retrospective nature. Consequently, we have introduced prospective, continuous audit and will present our preliminary findings of the introduction of voriconazole as primary prophylaxis. Furthermore, we will outline an ongoing study combining galactomannan, PCR and measurement of inflammatory markers in blood, broncho-alveolar lavage and exhaled breath condensate for the early diagnosis of invasive aspergillosis.

References


FUNGAL INFECTIONS DIAGNOSTIC IN A NECROPSY STUDY. COMPARISON WITH THEIR CLINICAL SUSPICION

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Background. Intensive therapies on haematology illnesses treatment can lead to an increase on fungal infection in patients and, occasionally, an aggressive medical profile. Samples that allows to establish the micro-
biologic or/and anatomopathologic diagnosis are not always easy to col-
lect, and frequently an empiric treatment has to be started, based only on a suspect diagnosis. Aims. To compare the correlation between the suspicion of invasive fungal infection (IFI) and its clinical manifestations with the findings of the autopsy, in patient with malignant haematol-
yogy diseases. Patients and Methods. We study 34 demised patients, 24 diagnosed patients of Acute Leukemia and 10 with other hematologic neoplasias that had been submitted to an autologous progenitor cell
transplantation. In 16 of the 34 patients the fungal infection was suspect-
ated at the beginning. According to the EORTC diagnostic criteria for IFI, 12 patients (75%), had a possible IFI and 4 cases (25%) presented a prob-
able IFI. There were no cases with a proven IFI before death. Results. The autopsy demonstrated the presence of fungal infection in 10 patients: in 7 cases there was a clinical suspicion of fungal infection while in three cases the infection was confirmed by histology. The organs shown up by the autopsy to be affected by the fungal infection were: lung (9 cases), digestive (6 cases), heart (2 cases), kidney (2 cases), CNS (2 cases) liver (2 cases) spleen (1 case), mediastinic mass (1 case), and pancreas (1 case). It is relevant that in most patients, the organic involvement oth-
er that lung was not suspected before their death, and it was responsi-
ble for their death. The autopsies have shown that fungal infection is related to the stage of the illness: superior vena cava syndrome (1 case), serious heart arrhyth-
mias (1 case), profuse diarrhea (1 case), renal failure (1 case), and hepat-
ic failure (1 case). Conclusion: Our study shows high incidence of clinical suspect IFI at the end-stage disease not confirmed with the autopsy, and the complexity of the clinical manifestations associated to this type of infections.

1253
CD40 LIGAND AND CALCIUM IONOPHORE TREATMENT OF DENDRITIC CELLS FROM
HEALTHY DONORS AND PATIENTS WITH MONOCLONAL GAMMAPathy OF UNKNOWN
SIGNIFICANCE AND MULTIPLE MYELOMA
L. Kovarova,1 M. Penka,2 R. Hajek2
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Backgrounds. Dendritic cells (DC) are the most potent antigen-presenting
cells that can initiate adaptive immune response. They can differen-
tiate from peripheral blood precursors and as an immature dendritic cells
they change their phenotypic, morphologic and functional characteristics.
Upon the activation/maturation process they differentiate from peripheral blood precursors and as an immature dendritic cells
they change their phenotypic, morphologic and functional characteristics.
They can differentiate from healthy volunteers and patients.
We studied the expression of TLR7, TLR8, TLR9, CCR7, CD38, CD54, CD63,
CD83, CD86, CD141, CD163, CD40, and CD1a in monocytes/macrophages and immature DCs from patients.
Results. 21 patients (1 case) with multiple myeloma (MM), 2 patients (1 case) with systemic light chain disease and 1 patient with plasmacytosis were studied.
Production of cytokines was determined after stimulation with CD40 ligand (CD40L) and calcium ionophore (CI).
Methods. Stimulation of DCs with different stimuli as CD40 ligand (CD40L) and calcium
ionophore (CI). Searching for differences in phenotype of DCs from healthy donors and patients.
Conclusions. Our study shows differences between donors and patients. Expression of HLA-DR was rel-
his because there were found no strong expression of CCR7, IL-12 and
production of cytokine IL-12 and CCL4 was increased in healthy donors. Production of cytokine IL-12 and
CCR7, IL-12, MIP-1α, HLA-DR. Results. The highest percentage of CD83,
characteristic marker of mature DCs, was found in 3rd day of culture
after stimulation with CD40L and also CI. In the 6th day was the average per-
centage of DCs positive for CD83 38.04% (38.04-78.23%), Cytotoxic and CD83
positive T cells was increased in vitro from 0.12×10⁶ to a median of 160×10⁶
(150×10⁶-420×10⁶) T cells within 4 weeks and the test of cytotoxicity has demonstrated a high degree of specific killing of ARH 77 myeloma cells 69.17% (58.41-82.98%). Cytotoxic activity has demonstrated only a modest specific killing of autologous mul-
tiple myeloma cells (18.8%) and allogeneic ARH 77 cells (18.2%). We have identified autologous and allogeneic myeloma-reactive T cells but only a modest effect in an autol-
ogous setting in patients with MM. Whether that is due to a low MACS
enrichment or low immunogenicity of autologous myeloma cell needs to be further clarified.
Funding. Supported by the grant: IGA MZ CR 14/8907-5.

1255
AMINO ACID SEQUENCES OF T CELL RECEPTOR REACTING AGAINST MULTIPLE
MYELOMA
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tute, BUCHAREST, Romania
Backgrounds. Multiple myeloma (MM) is a disease caused by malignant prolif-
eration of B lymphocytes in the bone marrow. Recently, high-dose chemotherapy with autologous hematopoietic transplantation has been considered a standard treatment for patients with advanced stages of MM. Such treatment delays relapse but it is curative and almost all patients ultimately develop recurrent disease. Based on preclinical and clinical studies it is evident that myeloma-reactive T lymphocytes play an important role in immunologic response to this malignant disease.
Myeloma-reactive T lymphocytes have been shown to be a promising approach in adoptive cellular immunotherapy aside autologous trans-
plantation of bone marrow graft. Aims. Our aim was to analyse T cell receptor (TCR) sequences reacting against multiple myeloma. Exper-
imental studies was performed in 10 patients to provide information on the specificity and spectrum of recognized antigens. Methods. Dendritic cells loaded with apoptotic bodies from magnetically isolated myeloma cells have been used to stimulate autologous T lymphocytes. Activated myeloma-specific T cells were identified and expanded. After mRNA iso-
dation the anchored reverse transcription using modified version of SMART method was done. PCR product was cloned into plasmid vec-
tor, transformed in bacterial cells and individual clones were sequenced. Results. Oligoclonality of TCR receptor was demonstrated in myeloma specific in vitro expanded T lymphocytes, in one case mono-
clonal population of tumor specific T cells was found. These findings support the assumption of myeloma specific antigens stimulating only certain autologous T lymphocytes. Conclusions. Structural characterization of TCR receptor of myeloma specific clones provides further evidence for the role of these T lymphocytes in immunotherapy. Receptor

1254
THE PREPARATION OF MYELOMA-SPECIFIC CYTOTOXIC T CELLS BASED ON INTERFERON-
γ PRODUCTION
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Backgrounds. Autologous hematopoietic stem cell transplantation has been considered recently as part of a standard treatment strategy in
patients with multiple myeloma (MM). Here we attempted to enhance the immunotherapeutic potential of autologous T cells based on selection of myeloma-reactive lymphocytes in vitro. Aims. The aim of this study was to identify and characterize autologous myeloma-reactive T cells in vitro and to evaluate their cytotoxic effect. Methods. Irradiated myeloma cell line ARH 77 or patient’s myeloma cells were used as tumor target cells. DCs were generated in vitro from mononuclear cells of 8 healthy volunteers and 10 MM patients were used for repeated stimulation of T lymphocytes. Activated T cells producing interferon γ were isolated using immunomagnetic separation (MACS) (Miltenyi Biotech) and expanded in vitro by phytohemagglutinin and high concentrations of interleukin 2. A specific cytotoxicity against myeloma cells was tested after the expansion with interleukin 2 and anti-CD3 and anti-CD28 antibodies. Activated T cells were labeled with CFSE. Allogeneic T cells and inter-
feron γ negative fraction of T cells served as controls. Results. In an al-
logeneic setting with ARH 77 cells the enrichment of interferon γ positive T cells by magnetic beads in healthy donors started from a median of
2.85% (1.97-4.58%) to 48.57% (15.14-92.98%) after MACS and from
1.91% (1.14-3.4%) to 73.14% (3.9-88.75%) after MACS in CD3+CD4+ and
CD3+CD8+ T cells, respectively. Interferon γ positive T cells were fur-
ther expanded in vitro from 0.5×10⁶ to a median of 160×10⁶ (150×10⁶-
420×10⁶) T cells within 4 weeks and the test of cytotoxicity has demonstrated a high degree of specific killing of ARH 77 myeloma cells 69.17% (58.41-82.98%). Cytotoxic activity has demonstrated only a modest specific killing of autologous mul-
tiple myeloma cells (18.8%) and allogeneic ARH 77 cells (18.2%). Con-
clusions. These data demonstrate a promising tumor-specific effect of
allogeneic myeloma-reactive T cells but only a modest effect in an autol-
ogous setting in patients with MM. Whether that is due to a low MACS
enrichment or low immunogenicity of autologous myeloma cell needs to be further clarified.
Funding. Supported by the grant: IGA MZ CR 14/8907-5.

1254
THE PREPARATION OF MYELOMA-SPECIFIC CYTOTOXIC T CELLS BASED ON INTERFERON-
γ PRODUCTION
D. Ocadiľkova1, L. Zahradova1, L. Kovarova,1 M. Penka,1 R. Hajek,1 J. Michalek,1
1LEHABI, BRNO, Czech Republic; 2Department of Clinical Hematology, BRNO, Czech Republic; 3Cancer Immunobiology Center, DALLAS, USA
Backgrounds. Autologous hematopoietic stem cell transplantation has been considered recently as part of a standard treatment strategy in
sequence determination can be used as a marker for evaluation of the vaccine strategy.

Funding: This work was supported by IGA MZCR 1A/8709-5.

1256
CRITERIA FOR CORD BLOOD DONOR SELECTION ON THE BASIS OF ROC CURVE ANALYSIS
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The main limitation factor for a wide use of umbilical cord blood (CB) for transplantation is the cell dose. In this sense, many cord blood banks have set a total nucleated cells (TNC) content ranging from 60 to 100 x 10^7 as minimum required values for storing the units. In order to optimise cord blood banking and reduce the number of UCB units deferred before processing, an effort in donor selection is mandatory. Many authors have showed that placental and neonatal weight influence hematopoietic content of cord blood units. To establish obstetric criteria for selection of cord blood units before cryopreservation. In order to determine the optimal placental and neonatal weight for selecting cord blood donors according to the number of TNC, we have performed Receiver Operating Characteristic (ROC) curve analysis. ROC curve is a graphical technique commonly used to find optimal cut off value of test using sensitivity and specificity data. We thought it could be useful to determine cut off values of placental and neonatal weight for an optimal selection of UCB units.

Table 1.

<table>
<thead>
<tr>
<th>TNC x 10^7</th>
<th>Cut-off</th>
<th>Area under the 95% confidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>60 x 10^7</td>
<td>Neutal weight ≥ 3190</td>
<td>0.63±0.013 0.616-0.653</td>
</tr>
<tr>
<td></td>
<td>Placental weight ≥ 646</td>
<td>0.68±0.013 0.666-0.704</td>
</tr>
<tr>
<td>70 x 10^7</td>
<td>Neutal weight ≥ 3195</td>
<td>0.63±0.012 0.619-0.656</td>
</tr>
<tr>
<td></td>
<td>Placental weight ≥ 645</td>
<td>0.68±0.012 0.662-0.701</td>
</tr>
<tr>
<td>80 x 10^7</td>
<td>Neutal weight ≥ 3195</td>
<td>0.63±0.011 0.614-0.651</td>
</tr>
<tr>
<td></td>
<td>Placental weight ≥ 635</td>
<td>0.67±0.011 0.656-0.695</td>
</tr>
<tr>
<td>90 x 10^7</td>
<td>Neutal weight ≥ 3195</td>
<td>0.63±0.011 0.612-0.649</td>
</tr>
<tr>
<td></td>
<td>Placental weight ≥ 635</td>
<td>0.64±0.011 0.629-0.668</td>
</tr>
<tr>
<td>100 x 10^7</td>
<td>Neutal weight ≥ 3195</td>
<td>0.62±0.011 0.605-0.642</td>
</tr>
<tr>
<td></td>
<td>Placental weight ≥ 635</td>
<td>0.63±0.011 0.617-0.657</td>
</tr>
</tbody>
</table>

Results. We revised 2590 cord blood units collected at Valencia Cord Blood bank for a four-year period. Mean TNC content of UCB before processing was 107.65 × 10^7. Mean TNC content of UCB before cryopreservation was 3313.36 × 10^7. Mean TNC content of UCB before cryopreservation was 543.7 g. and 652.2 g. Respectively and classification variables were considered placental weight and neonatal weight. Results are shown on the following Table. We conclude this statistical analysis can be helpful to determine cut off values of placental and neonatal weight for an optimal selection of UCB units.

1257
ANALYSIS OF THE CD34+ CELLS CONTENT OF THE CORD BLOOD UNITS STORED IN A REGIONAL CORD BLOOD BANK
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Some clinical studies have shown that graft selection should be based principally on CD34+ cell dose and grafts should contain at least 1.7 x 10^9 CD34+ cells per kilogram of recipient's body weight. However, criteria for selecting collections suitable for freezing and storage are not standardized. Although most banks have set a total nucleated cells (TNC) content ranging from 60 to 100 x 10^7 as initial minimum required values for storing the units, only a few banks selecting cord blood units on the basis of their CD34+ cell content. Aims. To analyse the CD34+ cell content of the cord blood units stored at the Valencia cord blood bank and the characteristics of the units according to their CD34+ cell content. We reviewed the data of 2149 cord blood units stored at Valencia cord blood bank and selected on the basis of their TNC content (more or equal than 100 x 10^7). CD34+ cells were quantified by flow cytometry. CB sample was taken directly from the bag and after volume reduction-before cryopreservation and 5 x 10^6 cells were incubated using monoclonal antibodies conjugated CD45 fluorescein and CD34 phycoerythrin (Becton Dickinson) and 7 amino-actomycin D as marker of DNA staining. Flow cytometric analysis was performed using CellQuest software. ProCount progenitor cell enumeration kit was used in comparison with our standard protocol, giving similar results. Total CD34+ cells content was calculated by multiplying the CD34 percentage per TNC. A total of 2149 cord blood units were stored for a 5 years period. Mean TNC, CD34+ cell percentages and total CD34+ cells were 112.37 × 10^7, 0.56 ± 0.25% and 41.79 × 10^7, respectively. From these units, 489 (22%) had a total CD34+ cell content less than 20 x 10^7. Characteristics of the units according to their CD34+ cell content are shown in the table. Conclusions. In order to increase the quality of cord blood units stored, the CD34 cell content should be considered as a selection criteria of cord blood units for cryopreservation and storing.

Table 1.

<table>
<thead>
<tr>
<th>CD34+ Content</th>
<th>% of Stored Units</th>
<th>CD34+ + TNC%</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 20%</td>
<td>489 (22.5%)</td>
<td>12.1±4.4%</td>
</tr>
<tr>
<td>20% - 40%</td>
<td>1111 (51.1%)</td>
<td>12.0±5.5%</td>
</tr>
<tr>
<td>40% - 60%</td>
<td>646 (29.6%)</td>
<td>14.3±1.5%</td>
</tr>
<tr>
<td>60% - 80%</td>
<td>646 (29.6%)</td>
<td>15.4±1.5%</td>
</tr>
</tbody>
</table>

1258
MESENCHYMAL STEM CELLS CONTRIBUTE TO THE HEALING PROCESS AND FUNCTIONAL IMPROVEMENT OF ISCHEMIC INJURED KIDNEY IN RAT MODEL
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Objective. Renal failure is a common disease with high morbidity and mortality. Ischemic injury is one of the most common cause of renal failure. Recent studies have reported that adult bone marrow-derived cells can contribute to renal remodeling and a dramatic repopulation of the mesangium. Moreover, there was a report that the role of bone marrow-derived hematopoietic stem cells in the regeneration of the renal tubular epithelium after ischemic injury in mice. When ischemic injury is inflicted on targeted organ, MSCs may migrate to the site of damage, undergo differentiation, and promote structural and functional repair. We evaluated whether bone marrow-derived MSCs contribute to the healing process and improve renal function in injured kidney of rat by ischemia. Materials and Methods. Right nephrectomy was performed in six-week-old SD rat. And the left renal artery and vein were clamped for 45 min followed by 2/3 nephrectomy was done and then clamp release to allow perfusion. MSCs prelabeled with green fluorescent protein (GFP) injected via tail vein. Peripheral blood was collected serially for evaluation of blood urea nitrogen and creatinine and functional evaluation was done with radioisotope renal scan. Histologic study and confocal microscopic evaluation were performed at 4 days, 1 week, and 4 weeks after MSCs injection. Results. We demonstrated that GFP positive cells were detected in damaged kidney by confocal microscopy and engrafted MSCs promoted healing process by ischemic injury. Also engrafted MSCs differentiated into tubular epithelial cells, thereby restoring renal structure. In the group with MSCs injection, the levels of blood urea nitrogen and creatinine were lower than control group without MSCs injection (BUN Day 4, control group; 65±0.81, MSC infusion group; 31±5.1). And MSCs injected rats demonstrated that renal func-
tion recovered more rapid and more close to the normal value in radioisotope renal scans. Conclusions: The results presented here suggest that MSCs are capable of healing and functional restoring of damaged kidney by ischemic injury. So MSCs may be useful for cell therapy of renal failure.

1259 IMMUNOREACTIVITY TO ANTI-FIBRONECTIN AND ANTI-LAMININ POLYCLONAL ANTIBODIES IN PARAFFIN-EMBEDDED MICE BONE MARROW ARE DEPENDENT ON HISTOPATHOLOGICAL PROCESSING

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Immunohistochemistry (IH) is an useful tool to study tissues and organs and it has been widely used in researchs or to supplement classical morphologic diagnosis including pathological conditions of the hemopoietic system. However, applicability of IH in bone marrow analyses presents some technical limitations, because some antigens are masked during tissue processing (fixation, decalcification and paraffin embedding) making the applicability of this methodology unfeasible. Bone marrow microenvironment contains cells of different tissues (bone, hematopoietic system and stromal elements) and several extracellular matrix (ECM) substances, mainly glycoproteins, proteoglycans and cytokines. The composition of bone marrow ECM is topographically variable and is associated to the development of different lineages of blood cells, suggesting the existence of specific interactions between ECM, stem cells and stromal elements. In previous studies, we have shown that bone marrow from mice submitted to protein malnutrition underwent structural changes with decrease in cellularity and increase of glycoproteins extracted from ECM. The aim of this work was to evaluate the influence of fixative and time of fixation in the antigenic preservation of extracellular matrix glycoproteins fibronectin and laminin, and their distribution in bone marrow of mice. Extremum of well nourished Swiss mice, 2 to 3 months old, were fixed with 3 different fixatives: Methacarn (1 hour), 10% neutral buffered formalin pH 7.2 (1 hour, 6 hour or 24 hours) and 4% buffered paraformaldehyde pH 7.2 (24 hours). Decalcified using 5% nitric acid (3 hours) or 10% buffered EDTA, pH 7.2 (7 days) and then processed routinely with standard dehydration and embedding in paraffin. Tissue sections (5 micron thick) mounted on silane coated slides were dewaxed, rehydrated, and brought to phosphate buffered saline. Endogenous peroxidase activity was blocked by incubation for 30 minutes in 3% hydrogen peroxide. Sections were incubated with primary antibodies against fibronectin (1:400) and laminin (1:25) overnight at 4°C. After washes in PBS, slides were incubated with biotinylated secondary antibody for 30 minutes and then processed routinely with standard dehydration and embedding in paraffin. Conclusions: The used conditions, adequate morphological and antigenic preservation of fibronectin and laminin were achieved on sections fixed in 10% buffered formalin during one hour and decalcified in 5% nitric acid. Tissue processing stages can significantly influence on immunoreactivity of antibodies against fibronectin and laminin. This way, sections fixed in 10% buffered formalin during one hour and decalcified in 5% nitric acid were selected to compare bone marrow ECM glycoproteins distribution in situ of nourished and malnourished mice.

Funding: Financial support: FAPESP, CAPES.

1260 VORICONAZOLE (VCZ) PROBABLY DOES NOT AFFECT THE PHARMACOKINETICS OF METHOTREXATE (MTX)

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Background and aim: Both MTX & VCZ have many drug interactions. Although the off-VCZ MTX levels of MTX differed significantly from those on-VCZ in the case, this reaction always resolved towards the next MTX. No side effects were observed, nor IPA did exacerbate under VCZ/MTX. Assessment of VCZ levels is planned. Conclusions: Although the off-VCZ levels of MTX differed significantly from those on-VCZ in the case, this could be attributed to the well known intra-patient inter-dose variability in MTX disposition. The bulk of data suggests that oral VCZ seems not to affect MTX pharmacokinetics significantly. However, this should be confirmed on a larger number of pts and/or doses of MTX given during VCZ therapy.

1261 CELL DIFFERENTIATION AND APOPTOSIS OF U-937 LEUKEMIA CELL LINES BY A NEW COMPOUND FROM DENDROSTALLERI Serratii

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Institute of Biochemistry and Biophysics, TEHRAN, Iran

Recently, we have reported on the activity of 3-hydrogenkwadaphine (3-HK), purified from Dendrostallera serratii, to induce differentiation and apoptosis in HL-60 cells upon a single dose treatment at a drug concentration of 5-20 nM. Regarding the relatively weaker potency of 3-HK compared to that of the crude extract, we looked for additional compound(s) with similar properties in the crude extract. Herein, we report on the isolation of a second and a more potent compound, with differentiation capability and apoptotic effects. The new compound inhibited growth and proliferation of U937 cells with an IC50 of 1.75 \mu M. The new compound, at 0.5-2.5 \mu g/ml inhibited proliferation of U937 cells by more than 70% and their viabilities were decreased by 47±2% after 72 h of treatment. The new compound also induced differentiation of U937 to monocyte/macrophage-type cells as became evident through phorbol ester-dependent reduction of NBT, morphological changes as examined by Wright-Giemsa staining and expression of CD11b and CD14 as analyzed by flow cytometry. The results indicated that treatment of U937 cells with the new compound for 3 to 4 days induced apoptosis as assayed qualitatively by acridine orange/ethidium bromide (Ao/Er) double staining, agarose gel electrophoresis and quantitatively by Annexin V technique using flow cytometry. Based on these observations, D. serratii could be a novel candidate for pharmaceutical evaluation.
and LLRBC(L) hereinafter referred to as U and L). Then, each layer of blood cells is put into 3 mL of RPMI-1640 solution and cultured in a 37°C 5%-CO2 incubator. Assuming that the entire amount of daily dosage of drug administered to the patient (Q) is absorbed in 5 L of blood, the absorbed amount of drug in 3 mL of blood (X) is calculated as follows: X = (Q x 3 mL) / 5 L, i.e. X = 0.3 x Q / 5.000. The calculated absorbed amount of drug (Q) is added to 3 mL of physiological saline, and fully mingled together. The solution is diluted with the plasma, which the drug is dissolved, is sterilized by filtering in a clean bench. The sterilized solution is put into U and L, and cultured in a 37°C 5%-CO2 incubator. They are diachronically monitored with an inverted phase-contrast microscope, and recorded in photos and VCRs. U and L which the drug-dissolved solution is not added to are used as the control groups. Results. Compared with the control groups, if the drug is harmful for the red blood cells, RBC shows deformation and degeneration earlier, and dies after getting into the cells like brightly, ghost and black shell; i.e., the life of RBC is shortened. The WBCs (white blood cells) grow to enormous size in various complicated shapes, staying alive for three to five weeks. Moving Micro Living cells and Mysterious chains emerge in some cases. Compared with the control groups, Photo-Cytosis Phenomenon (named by Matsumoto) occurs sooner in some cases and later in other cases. Conclusions. If the proposed method is clinically adopted, individual variation against side effect of drugs can be determined early. It would be secure and risk-free to check all of the drugs administered before adulthood. I believe medical science in the 21st century will go on in this direction. If the proposed method is started to use at the point of drug development, it can cut down on waste and expenses drastically.

1263 UNEXPECTED SUBACUTE LEUCOENCEPHALOPATHY FOLLOWING INTRATHECAL METHOTREXATE AND CYTARABINE ADMINISTRATION IN A PATIENT HOMOZYGOUS FOR MTHFR 677C→T POLYMORPHISM

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Background. Intrathecal administration (ITA) of chemotherapy, mainly Methotrexate (MTX) and Cytarabine (Ara-C), is a standard approach to central nervous system (CNS) prophylaxis of aggressive Non Hodgkin Lymphomas. MTX is known to cause diffuse symmetrical leucoencephalopathy in children. A genetic variant of Methylene-tetrahydrofolate reductase (MTHFR) due to the 677C→T polymorphism determines a striking reduction in the enzyme activity and has been associated with increased toxicity during MTX administration in children. Reports on toxic effects in adults are lacking despite the increasing use of aggressive protocols in this age group. Aim. To report the case of an adult, carrying the MTHFR homozygous mutant 677TT genotype, who developed subacute leucoencephalopathy following intrathecal prophylaxis with MTX and Ara-C. Case report. A 82-year-old Caucasian woman diagnosed with aggressive B-cell NHL (stage IV B, bone marrow, liver and spleen involvement). Treatment was planned according to modified POG 8617 regimen (Todeschini G, Ann Oncol, 1997). Course A included CNS prophylaxis with intrathecal MTX (12 mg) and Ara-C (50 mg) at days 1 and 4. CSF biochemical examination was normal and cytospin was acellular. Six days after day 4 ITA, the patient became acutely confused and showed behavioural and speech disturbances. Motor impairment of the right leg was also recorded. Psychiatric assessment excluded a psychiatric origin for this disorder. Body temperature was normal and he had no signs of meningal involvement. No signs of infection were found. Brain CT and MRI were unremarkable. EEG showed predominantly frontal disturbances. Brain MRI performed 109 days after day 4 IT, showed bilateral hyperintense lesions in subcortical white matter in T2-weighted images. The molecular analysis detected a MTHFR 677TT homozygous mutant genotype. Discussion. The present case report indicates the possible event of severe CNS damage in adults undergoing ITA administration of MTX. The mechanisms underlying MTX toxicity remain uncertain. Review of possible mechanisms: Methotrexate is metabolized to 7-OH-MTX, which competes with methylenetetrahydrofolate for several metabolic pathways affected by the drug. MTHFR is crucial to folate metabolism, essential for DNA synthesis and repair pathways as well as DNA methylation and its severe deficiency results in hyperhemo-cysteinemia, which can cause neurotoxicity. Although we cannot completely rule out the possible role of cytara dine in causing CNS toxicity in our patient, the clinical and radiological findings suggest the major role of methotrexate. Ara-C neurotoxicity is mainly reported in children and there is a preferential involvement of the spinal cord rather than the brain. The presence of the TT homozygosity in our case seems to confirm the predisposing role of this genotype for CNS damage in IM and might help to clarify the reasons for the high prevalence of the homozygous 677TT genotype in the Italian population, ITA MTX prophylaxis should be carefully followed-up and leucovorin rescue in adults should be considered.
9.3 and 10.8 IU/gHb. The infant started on daily oral methylene blu (3 mg/kg/die) and methemoglobin level remained < 7%. Molecular analysis of DIA1 gene showed the presence of the new homozygous missense mutation GCC-GAC at codon 143, resulting in the aminoacidic substitution Gly143Asp. The mutation was present in parents at the heterozygous level. Consanguinity was denied but it can be supposed since the parents were the same little enclave. 

Case 2: a 6-month-old boy, was born at term as second child of consanguineous Egyptian parents (1 grade cousins of RGM type II: cyanosis associated with profound mental retardation, microcephaly, bilateral athetosis, strabismus, frequent vomiting and crying. Studies on RBC revealed marked NADH-cytb5r deficiency (1.4 IU/gHb) versus his mother father and healthy siblings at levels of 10.1, 5.8 and 10.7 IU/gHb respectively. Molecular analysis of DIA1 gene showed the new homozygous intronic mutation IVS2+2 c-t which probably results in splicing alterations and absence of protein functional, and may therefore account for the severe clinical pattern.

**1266**

CELL CYCLE STATE OF HEMATOPOIETIC PROGENITOR CELLS AND BONE MARROW LYMPHOCYTE PHENOTYPE IN PATIENTS WITH ACQUIRED APLASTIC ANEMIA

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¹Institute of Clinical Immunology, NOVOSIBIRSK, Russian Federation; ²State Medical University, NOVOSIBIRSK, Russian Federation

**Backgrounds.** Acquired aplastic anemia (AA) is associated with profound quantitative and functional defects in the hematopoietic stem cell compartment and cell-mediated suppression. Aim. The aim of the study was to evaluate cell cycle state of hematopoietic progenitor cells and phenotype of bone marrow lymphocytes in patients with AA during immunosuppressive treatment (IST) and after allogeneic bone marrow transplantation (alloBMT). Methods. The study was performed in 38 patients (19 male, 14 female) including 9 with non-severe AA (NSAA), 19 with severe AA (SAA) and 5 with very severe AA (VSAA). There were 5 cases of hepatitis-associated AA and 2 cases of pregnancy-associated AA. In 26 cases, no cause of AA was identified. The median age at diagnosis was 20 years (range, 13-55 years). Combined IST with repeated courses of horse antithymocyte globulin (ATG) and cyclosporine A (CsA) was used for 19 patients with SAA and VSAA. Nine patients with NSAA were treated with CsA alone. AlloBMT from related matched sibling was performed in 4 young patients with SAA/VSAA. The phenotype of BM lymphocytes and the number of CD34-positive and HAE-9-positive erythroid cells were determined by single-color and double-color flow cytometry analysis. Cell cycle and apoptosis of CD34+ and HAE-9+ cell populations were studied by using the 7- amino actinomycin D (7AAD). As controls 14 healthy age-matched volunteers were studied. Results. The overall survival is 94% at the median follow-up of 30 months (range, 6-96 month). Complete or partial remission was achieved in 20 patients in IST group. AlloBMT of patients are in complete remission. The numbers of CD34+ and HAE-9+ BM cells were decreased in most of untreated AA patients (2-3 fold below normal). In control group the mean number apoptotic CD34+ cells was 1% and 81% of cells were in G0/G1 phase of cell cycle. In more than 50% of patients were decreased in most of untreated AA patients (2-3 fold below normal).

**Conclusion**. Modern treatment modalities provide hematological recovery was more complete in patients after alloBMT than after IST. The number of CD8 cells are highly restricted CD8+ lymphocytes significantly increased in BM during active phase of AA and returned to normal level at 6 month after ATG treatment. However the number of CD8+ lymphocytes increased again in most of patients after 12 month follow-up. Conclusion. Modern treatment modalities provide hematopoietic response and long-term survival in more than 80% of AA patients. Our data confirm that increased apoptosis and replicates stress in CD34+ cell compartment with profound stem cells deficiency correlate with signs of T-cell activation process in AA. Assessment of hematopoietic reservoir and immune-mediated pathology may provide additional information about remission status in AA patients.

**1267**

IL-12 AND IL-10 PRODUCTION BY DENDRITIC CELLS (DC) FROM PATIENTS WITH APLASTIC ANEMIA (AA)

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DCs accomplish determining function in antigen-specific T-cell immune response and antigen-specific self-tolerance development. Moreover, they yield either Th1 or Th2 commitment of naive lymphocytes due to immunoregulatory cytokines (IL-10 and IL-12) production. As AA is characterized with increased Th1/Th2 ratio and Th1-mediated suppression of hematopoiesis, DC maturation profile for HLA-DR2 restricted T-clone activation in AA patients. However, functional peculiarities such as IL-10 and IL-12 production by DC in AA patients still remain to be elucidated. Therefore, IL-12 and IL-10 secretion capabilities of DC generated from peripheral mononuclear cells (PMNC) of 25 AA patients before and after the immunosuppressive therapy (IST) were compared with DCs of 4 healthy donors (Hs). DC were derived from PMNC in the presence of GM-CSF and IL-4 for 5-7 days and further stimulated for 48 hours by exposition either to LPS or to CD40L expressing cells. Supernatants of DC cultures were tested by ELISA for IL-12 and IL-12 production, respectively. DC of 78.6% (17/25) of AA patients showed significantly lower levels of IL-10 production at baseline in comparison with control group data. 1st stage of IST resulted in a tendency of IL-10 production enhancement, though to lower amounts than that of Hs. Considerable increase in the level of IL-10 production by DCs of AA patients after durable IST correlated with achievement of partial or complete remission. Moreover, level of IL-10 production by DCs of AA patients in remission exceeded that of DCs of Hs (224 vs 166 pg/106cells, respectively). 36.4% (4/11) of AA patients exhibited increased baseline levels of IL-12 production by DCs compared to those of Hs. These patients appeared to be resistant to standard scheme of IST and required continuous IST including repeated courses of antithromocyte globulin and cyclosporine-A. However, significant diminution of IL-12 production by DCs of AA patients after 1 stage of IST was associated with favorable outcome. Patients that had shown low initial levels of IL-12 secretion by DCs comprised the best prognostic group. These data suggest that dysregulation of IL-10 and IL-12 production by DCs of AA patients might contribute to autocrine T-clone expansion and consequently, to AA development.

**1268**

INHERITED BONE MARROW FAILURE SYNDROMES IN LEBANON. PILOT DATA FROM THE LEbanese PEDIATRIC HEMATOLOGY/ONCOLOGY GROUP

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**Backgrounds.** Inherited bone marrow failure syndromes (IBMFS) are rare disorders of the bone marrow with an increased risk of malignancy. Genetic features of these disorders are still being studied and could vary among different ethnic groups. Although some data is available from registries in the US and Europe, there is no such data from the Middle East and particularly from Lebanon. Aims. We aim to study the incidence, outcome and overall condition of patients affected with these disorders in Lebanon, as well as the genetic features of their families in order to establish a National IBMFS registry. Methods. Patients with the following diagnosis were included: Amegakaryocytic Thrombocytopenia, Diamond-Blackfan anemia (DBA), Dyskeratosis Congenita, Fanconi’s Anemia, Pearson’s Syndrome, Severe Congenital Neutropenia, Shwachman-Diamond Syndrome, Thrombocytopenia Absent Radii (TAR). Data collection sheets were filled by the pediatric hematologist for each patient diagnosed with any of these disorders between 1970 and 2006. Data was later entered into an Excel workbook and statistical analysis was performed. Results. Forty two (42) patients were identified so far. Fourteen had Fanconi anemia, nineteen had DBA, and six had severe congenital neutropenia, one had TAR syndrome, one had amegakaryocytic thrombocytopenia and one had Shwachman-Diamond Syndrome. Mean age was 10.2 years. At the time of data collection, 67% were alive and 33% were dead. Death was due to malignancy in 6 out
of 14 cases. DNA was available in 11 patients and was studied for pos-
sible mutations in the disease specific genes. Further results will be pre-
sented at the meeting. Summary/Conclusions. This is the first study look-
ing at Inherited Bone Marrow Failure Syndromes in Lebanon. A registry
has been created and is being updated constantly with new cases. A
larger regional registry should be created with collaborative efforts and
data should be compared among countries and then to registries in
Europe and the USA in order to improve diagnosis and outcome of these
patients and compare genetic determinants of these complex disorders.

1269
ALLOGENEIC TRANSPLANTATION IN ADVANCED STAGES OF CML IN THE THIRD
MILLENNIUM. IS THERE A DIFFERENCE ?
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Although since 2001 thyrosine kinase inhibitors have substantially
changed the therapeutic approach in CML (chronic myelogenous
leukaemia), its advanced stages still remain an important problem,
in which transplantation is considered. Methods. We evaluated retrospect-
vatively 36 patients transplanted between the years 1992-2005 in the
advanced stages of CML (i.e. further than 1st chronic phase). 20 of them
received the treatment in the nineties (non-imatinib era)(group 1),
while 16 patients were transplanted in the last 5 years (group 2). In these
patients imatinib was used either before transplantation (12 pts, 4 of
them progressed despite this treatment), or after the transplantation (6
pts). Differences in disease stage when entering the transplantation,
transplant related mortality, overall survival, relaps rate and its response
to further treatment were evaluated. Results. While in the group 1 there
were only 25% of patients entering the transplantation in the re-
achieved chronic phase, the number increased to 44% in the group 2.
The median of overall survival was 1.5 months in the group 1
compared to 16 months in the group 2. There are 3 surviving patients (15%)
in the group 1 and 11 (65%) in the group 2. There was an enormous 100 days
mortality in the group 1 (3 pts, i.e. 65%) compared to absent 100 days
mortality in the group 2. Remission was achieved in 6 patients in the
group 1, (3 of them relapsed later) and in 14 patients in the group 2, (6
of them relapsed later). DLI (donor lymphocyte infusion) or next trans-
plantation was used as a relaps treatment in 2 patients in the group 1 and
5 in the patients in the group 2. In 6 patients of the group 2 imatinib was
used after transplant. Conclusions. More feasible and probably less toxic
achievement of further chronic phase by chemotherapeutic combina-
tions with imatinib, better supportive care, earlier detection of minimal
residual disease or relaps by molecular techniques, the use of imatinib for
post remission treatment were found to be more contributive factors for
better survival in patients transplanted in the advanced stages of CML.

1270
INFECTION TRANSMISSION DURING GRAFT IN STEM CELL TRANSPLANTATION SYSTEM
OF PREVENTION
M. Blaha, P. Mericka, J. Maly, L. Jelavy, P. Zak, M. Cermanova,
S. Filip, M. Blazek, R. Maly, V. Vehracek
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Backgrounds. The possibility of infection transmission by transplanta-
tion of cryopreserved blood stem cell concentrates is well known. For
this reason EBMT (European Blood and Marrow Transplantation Group)
and ISHAGE (International Society for Haemotherapy and Graft Engi-
neering) standards include a panel of serological tests to be performed
directly before the transplant in the donor and/or recipient, in order to
minimize the likelihood of infection transmission. Aim: In the submitted paper attention is focused on dan-
ger of infection transmission by infusion of cryopreserved peripheral
blood progenitor cells or bone marrow to the patient and/or cross con-
tamination of stored grafts. Methods. During the preliminary investiga-
tions published 3 years ago the study was performed on a group of 35
related donors for allogeneic transplantation and 152 pts (mal.lym-
homas, multiple myel., leukemias, solid tumors, amyloidosis). They
were tested before the peripheral blood stem cell or bone marrow harv-
vest according to the standards of EBMT and ISHAGE-Europe: retro-
viruses (HIV,HTLV), hepatitis (A, B, C), herpes viruses (CMV, EBV, VZV,
HSV), and others. Results. Laboratory signs of active infection
were found in 26 donors (62,85%) and in 91 patients (59,9%). The
active infection from herpes viruses was the most common - in patients
50, in donors 21. Hepatitis B was found in only two cases. Conclusion:
We can conclude that the rate of clinically unsuspected (but dangerous)
infections in donors and patients remains relatively high in spite of the
fact that the system of donor search and the whole transplantation pro-
cedure have improved in the last years. We confirmed that the developed
system of safety assurance is extremely important and that the whole
package of preventive tests according to EBMT and ISHAGE remains ful-
ly justified.

1271
EVOlUTION OF PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA (ET) TREATED WITH
HYDROXIUREA (HU) and α - INTERFERON (IFN)
A. Colita,1 A.R. Lupu,1 N. Berbeci,2 G. Mocanu,3 S. Angelescu,3
D. Barbu,3 C. Vlaicu,3 S. Crin Scotia,3 C. Ciocan,3 M. Closca,3
A.M. Vladareanu,1 H. Bumbea,2 D. Mut Popenescu2
1Coltca Clinical Hospital/UMF Carol Davila, BUCHAREST, Romania; 2Colc
Hospital, BUCHAREST, Romania; 3University Hospital/ UMF Carol Davila,
BUCHAREST, Romania
Backgrounds. Essential thrombocythemia (ET) is a clonal myelo-
 proliferative disease characterized by increased number of platelets,
megakaryocytic hiperplasia and tendency to thrombosis and/or hemor-
rhage. The major aim of therapy is preventing thrombotic and hemor-
 rhagic complications using cytoreductive agents that do not increase the
risk of progression to acute leukemia or myelofibrosis. Aim of the study,
was to compare the results of the therapy with HU versus IFN in ET patients.
Patients and Methods. 72 patients with ET followed between June 1999
- July 2005; median age 59,5 years (range 33 - 87 years); M/F: 32/40.
Diagnostic criteria were those of the PVSG. 44 patients (median age 62
years) received HU, and 28 patients (median age 51 years) received IFN.
HU dosis varied according to platelet counts between 500 and 1500
mg/d. IFN dosis was 9 MU/week. The purpose of therapy was to
maintain platelet counts below 600.000/cmm and to prevent thrombot-
 ic or hemorrhagic complications. Patient with high risk for thrombosis
received aspirine (75 mg/zi). Results. Patients treated with IFN present-
ed a 90% response rate and those receiving HU had a 75% response rate.
The reduction of platelet counts below 600.000/cmm was faster in the
HU group versus IFN group (average 4 weeks vs 10 weeks respective-
ly). The level of platelets during the treatment was maintained constant-
ly around 400.000/cmm with IFN whereas in the HU group it was
around 600.000/cmm. Thrombotic complications occurred more often in
the IFN group - 8 cases (28,5%) with predominance of arterial throm-
 bosis - 6 cases. In the HU group the incidence of arterial thrombosis was
18,18% (3 cases - arterial thrombosis). The treatment with HU was better tol-
 erated 6 cases with reversible leucopenia. The therapy with IFN was
worse tolerated - 4 patients abandoned the treatment because of gener-
 al symptoms. Conclusions. IFN was more effective in assuring a rapid and
constant decrease of platelet number, but in the HU-treated group there
was a lower incidence of thrombotic complications.

1272
CHRONIC MYELOPROLIFERATIVE DISORDERS: USE OF WHOLE BLOOD PLATELET
LUMI-AGGREGOMETRY (WBPA) TO OPTIMISE ANTI-PLATELET THERAPY IN PATIENTS
WITH PLATELET HYPERACTIVITY
A. Manoharan, R. Gemmell, T. Hartwell
St. George Hospital, SYDNEY, Australia
Twenty seven patients with chronic myeloproliferative disorders and
including evidence of platelet hyperactivity on WBPA studies (Sr] Hema-
tol 199:105:618) were commenced on anti-platelet therapy comprising
aspirin, clopidogrel and/or odourless garlic and the studies were repeat-
ed to assess the efficacy of the therapeutic agents. Only eight patients
showed clear evidence of anti-platelet effect (inhibition of aggregation
with arachidonic acid; aggregation and disaggregation with ristocetin),
whereas the aspirin (100 mg/d) was tolerated in all. After 3 months 13
patients required a higher dosage of aspirin and/or an additional
anti-platelet agent to achieve therapeutic efficacy. Lumi-aggregometry
also proved useful to optimise therapy in the six patients who received
clopidogrel (reduction in response to ADP) or odourless garlic (change of
overall platelet function from hyper-activity to normal or hypo-activity),
whereas the aspirin intolerance. Conclusions. Our experience suggests that
WBPA studies will not only enable selection of patients who will bene-
fit from anti-platelet therapy but also assess the efficacy of such therapy.
Study the prevalence of the point mutation V617F on samples obtained from bone marrow aspirates, or peripheral blood, suffering from CMPD, and to determine its potential contribution for the distributed as follows: 3 on the group diagnosed as reactive myeloid cytoses. From the total of patients studied (99), 53 were JAK2+ (53.5%), and distributed as follows: 3 on the group diagnosed as reactive myeloid cytoses. This derives, in part, because CMPD are frequently difficult to diagnose due to the considerations of the diagnosis. 4) The screening for the JAK2 mutation was performed on samples obtained from bone marrow aspirates, or peripheral blood, using PCR according to E. Joanna Baxter technique (Launet 2005). Results. From the total of patients studied (99), 53 were JAK2+ (53.5%), and distributed as follows: 3 on the group diagnosed as reactive myeloid cytoses (18.7%), 4 on the non confirmed CMPD (28.6%), and 46 on the CMPD (66.7%). The JAK2: mutation’s prevalence on the different subgroups of CEMP: was 15 (58.6%) of 28 for ET, 13 (92.3%) of 14 cases of PV, 3 (60.0%) of 5 cases of CVM and 15 (62.2%) of 22 patients with mixed CEMP. Conclusions: Allele-specific PCR is an effective method for the detection of JAK2: mutation with no need of a mutation screening. 2) Inside the CEMP, the highest percentage of JAK2: patients belonged to the PV group. 3) The percentage of JAK2: patients on the mixed CEMP is also high, which could mean that among these patients some PV cases could still remain incorrectly diagnosed. 4) The screening for the JAK2 mutation was useful to diagnose CEMP on patients which had been previously diagnosed as reactive myeloid cytoses. This derives, in part, because CEMP are frequently difficult to diagnose due to the considerable clinico-pathologic overlap with reactive cytoses.

Prevalence of the activating JAK2 tyrosine kinase mutation V617F in Taiwanese patients with myeloproliferative disorders

Taipei-Veterans General Hospital, Taipei, Taiwan; National Yang-Ming University, Taipei, Taiwan

Background. The JAK2 V617F mutation has been recently reported in patients with polycythemia vera(PV) and a proportion of patients with essential thrombocythemia(ET) and myelofibrosis with myeloid metaplasia(MMP). This acquired point mutation constitutively activates JAK2 tyrosine kinase and is believed to underlie growth factor hypersensitivity displayed by hematopoietic progenitors in these disorders. Patients and Methods. In this study, allele-specific PCR (ASPCR) was performed with a primer pair common to both wild-type and mutant alleles. We amplified a JAK2 exon 12 fragment from peripheral blood leukocyte from 96 patients by ASPCR followed by digestion with BsaXI restriction endonuclease (PCR-RFLP). The JAK2V617F mutation abolishes a BsaXI restriction site present in the wild-type sequence and generates a different band pattern. Results. JAK2 mutation could be detected in 25 of 31 PV patients (80.6%), 23 of 38 ET patients (60.5%), 2 of 6 MF patients (33.3%), none of 11 MDS patients and 10 patients with other diseases. There is no significant difference between JAK2 mutation positive and negative patients in the age, peripheral blood counts, creatinine and antecedent cancer history. The thrombotic or cerebral bleeding complication occurred in 15 out of 48 patients with JAK2 mutation (+), but only 4 of 26 patients with JAK2 mutation (-)<(27% vs. 16%; p = 0.44). Conclusions. JAK2 V617F mutation can be frequently detected in the Taiwanese patients with myeloproliferative disorders as in the western patients, which should be used as a diagnostic tool in the future routine hematological practice.

EPOR mutations in familial congenital polycythemia vera

Hematology Clinic, IOANNINA, Greece; Laboratory of Genetics Unit, IOANNINA, Greece

Background. Primary familial congenital polycythemia (PFCP) is a rare myeloproliferative congenital and dominant disorder. It is caused by inherited defects in hypoxia sensing or by inherited vital defects in red blood cell precursors that cause augmented responsiveness to Epo. These facts result in isolated proliferation of the erythroid progenitor cells and in erythrocytosis. The Epo-receptors (Epo-R) is located on the surface of erythroid cells. The Epo-R gene is situated on chromosome 19. Genetic changes of the Epo-R gene have been related to the pathogenesis of PFCP. Specifically, twelve mutations in patients with PFCP have been recognized. Aim of this study was to investigate the presence of Epo-R mutations in patients with PFPC. Methods. We studied eight families (20 individuals) with PFCP of Greek origin. All individuals had Hb>15% and their age range was between 5 and 58 years, 3 children and 16 adults. Genomic DNA was extracted from peripheral blood lymphocytes, according to standard procedures. SSCP and sequencing analysis was performed to detect mutations in exon VIII of the Epo-R gene that previously has been related to PFCP. Results. No mutation was identified in our patients which could underlie the molecular defects of the PFCP. Conclusions. Our results can lead to the conclusion that the molecular cause of familial polycythemia in Greek patients cannot be attributed to sequence alterations in exon VIII of the Epo-R gene.

HLA associations with child’s and adult’s acute lymphoblastic leukemia

E. Khmaganova, L. Murashova, O. Korovina, Y. Zaretskaya
Research Center for Hematology, MOSCOW, Russian Federation

Background. Acute lymphoblastic leukemia (ALL) has two rising of morbidity: in children of 2-4 years and in adults after 50 years. The aim of our study was to identify the associations between HLA and ALL in children and in adults in our population.

Methods. HLA-A, -B, -CM, DRB1 typing was done in 26 children with ALL (the median patient age was years 5.6; age, 1-13 years) and in 42 adult patients with ALL (the median patient age was 32.6 years; range, 16-52 years). 328 healthy donors of blood components (the median age was 29.9 years; range, 18-59 years) were control group. The HLA frequencies were counted and compared by exact Fisher’s test. The strength of association between HLA and disease was determined by the evaluation of relative risk (RR). Results. Children with ALL had significantly increased frequency of DRB1*07 (46.2% vs. 26.8% in control group, see the table below), RR of child’s ALL for DRB1*07 carriers was 2.5. Adults with ALL had significantly increased frequency of DRB1*11 (38.1% vs. 22.6% in control group). RR of adult’s ALL for DRB1*11 carriers was 2.1. The frequency of DRB1*01 was significantly decreased in both groups: in children with ALL (7.7%) and in adults with ALL (11.9%) in comparison with controls (26.2%). RR of child’s and adult’s ALL for DRB1*01 carriers in our population was 0.23 and 0.38 respectively. In conclusion, it seems that DRB1 gene may be involved in predisposition and resistance to ALL development both in children and adults.

<table>
<thead>
<tr>
<th></th>
<th>Children with ALL</th>
<th>Adults with ALL</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=328</td>
<td>n=26 n=42</td>
<td>n=328</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>II</td>
<td>III</td>
<td></td>
</tr>
<tr>
<td>DRB1*01</td>
<td>7.7</td>
<td>11.9</td>
<td>26.2</td>
</tr>
<tr>
<td>p&lt;0.05</td>
<td>p&lt;0.01</td>
<td>p&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>DRB1*07</td>
<td>46.2</td>
<td>21.4</td>
<td>26.8</td>
</tr>
<tr>
<td>p&lt;0.05</td>
<td>p&lt;0.05</td>
<td>p&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>DRB1*11</td>
<td>34.6</td>
<td>38.1</td>
<td>22.6</td>
</tr>
<tr>
<td>p&lt;0.05</td>
<td>p&lt;0.05</td>
<td>p&lt;0.05</td>
<td></td>
</tr>
</tbody>
</table>
1277
INTERMEDIATE ANALYSIS OF THE SPANISH QUIT REGISTRY FOR PATIENTS WITH ACUTE LEUKEMIAS AND NON-HODGKINS LYMPHOMAS TREATED WITH INTRATECHAL CHEMOTHERAPY

Background and Aim. CNS involvement in pts diagnosed with hematological malignancies is an unfrequent complication that carries poor prognosis. The indication and the schedules of prophylaxis and treatment of CNS involvement in AL and NHL are not homogenous within countries and within the same country. The aim of the QUIT study was to report the practices of CNS prophylaxis and treatment in patients with AL and NHL in Spain. Methods. Prospective study conducted from June to December 2005. Adult pts (≥18 yr.) diagnosed with hematological malignancies who received IT chemotherapy as CNS prophylaxis or treatment were consecutively included through online registration. Results. 242 pts from 27 hospitals included 172 NHL and 70 AML. CNS prophylaxis: 15/24 patients (62%), IT liposomal depot cytarabine in 8/24 (33%) and MTX+ARAC+Hydrocortisone) in 14 and liposomal depot cytarabine in 8/24 (33%). IT therapy consisted of triple IT therapy (TIT, MTX+ARAC+Hydrocortisone) in 14 and liposomal depot cytarabine in 3 pts. CNS prophylaxis: 125 patients and consisted of TIT in 104 (83%), and MTX IT in 19 (15%), IT ARAC in 1 (1%) and 1 IT liposomal depot cytarabine (1%). No cranial irradiation either for prophylaxis or therapy was given in any case. 2. NHL patients: 56 diffuse large B-cell, 18 Burkitt’s, 5 follicular, 5 mantle cell, 10 T cell, and 6 other subtypes; stage IV 70%, B symptoms 52%, bulky disease 51%, extranodal involvement 79% (bone marrow 45%) and >1 extranodal site 44%, increased LDH levels 64%, IPI score ≥3 (31%), bulky disease 31%, extranodal involvement >1 extranodal site 44%, increased LDH levels 64%, IPI score 3 68%. CNS therapy: 24 pts, 16 at diagnosis and 8 as first (5) or subsequent relapses (3). CNS therapy consisted of TIT in 88% followed by MTX IT in 9% and IT liposomal depot cytarabine in 3%. Cranial irradiation was administered in 3 cases (2 as therapy an 1 as prophylaxis). Conclusions. In clinical practice in Spain the patterns of CNS prophylaxis and therapy for AL are homogeneous. For NHL there is heterogeneity of indication of prophylaxis. TIT was the most frequent schedule for CNS prophylaxis and therapy in AL and NHL. It is of note the administration of new drugs i.e. liposomal depot cytarabine for CNS therapy and prophylaxis in NHL and AL, and the lack of use of cranial irradiation.

1278
COMPARISON OF METHODS FOR DETERMINING ζ-CHAIN ASSOCIATED PROTEIN 70 (ZAP-70) EXPRESSION IN PATIENTS WITH B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA (B-CLL)
K.G. Giannopoulos,1 A. Bojarska-Junak,2 M. Kowal,1 A. Dmoszynska,1 J. Rolinski1
1Medical University of Lublin, LUBLIN, Poland; 2Dept. of Clinical Immunology, LUBLIN, Poland

Background and Aims. ζ-chain associated protein of 70kDa (ZAP-70) is the most promising surrogate marker for the IgVH mutation status. ZAP-70 is a signaling molecule from Syk/ZAP-70 protein kinase family normally utilised by T and NK cell and abnormally expressed in B-CLL cells. Expression of ZAP-70 protein in B-CLL detected by flow-cytometric analysis, correlated with IgVH mutational status, disease progression as well as survival and revealed even better prognostic value compared to IgVH mutation. Crespo et al. proposed the method of ZAP-70 detection by flow cytometric test. Recently several novel monoclonal antibodies appeared on market. This paper we compared different methods of determining ZAP-70 expression in B-CLL. We wanted to find most clinically relevant and easy assay to determine ZAP-70 expression in B-CLL. Methods. We compared different clones of monoclonal antibodies against ZAP-70 with direct and indirect staining, ZAP-70 expression utilizing whole blood protocol and peripheral mononuclear cells isolated from whole blood and additionally the use of different reagents for permeabilization. Results and Conclusions. Basing on results obtained during this study we recommend use of anti-ZAP-70 PE, clone 1E7.2 monoclonal antibodies utilizing whole blood protocol as an easy method that brings completely compatible results to the original method proposed by Crespo et al.

1279
EXPRESSION OF ZAP-70, CD38 AND IGVH MUTATIONAL STATUS AS PREDICTORS OF TREATMENT IN BINET STAGE A CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)
Hospital Clinico, VALENCIA, Spain

Backgrounds. A combination of Zap-70/CD38 expression has been proposed as a predictor of treatment in CLL patients by several authors. Aim: to analyse the predictive value of this model in a series of 73 patients consecutively diagnosed of B-CLL at our institution from 1997 until 2005, whose all three variables were available. Methods. Zap-70 and CD38 expression was analysed by flow cytometry and IgVH mutational status by direct sequencing with 98% cut off. All 73 patients were in Binet A. Median age was 66 years (54 to 85), male sex 40 (52%). ZAP-70 and CD38 expression cut off were 20% for both antigens. Median follow-up was 56 months (6.7 to 134).

Table 1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>N(%)</th>
<th>Time to treatment</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD38</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20%</td>
<td>48 (66)</td>
<td>25 (34)</td>
<td>0.013</td>
</tr>
<tr>
<td>≥20%</td>
<td>25 (34)</td>
<td>42.9</td>
<td></td>
</tr>
<tr>
<td>ZAP-70</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20%</td>
<td>44 (60)</td>
<td>29 (40)</td>
<td>0.013</td>
</tr>
<tr>
<td>≥20%</td>
<td>29 (40)</td>
<td>48.3</td>
<td></td>
</tr>
<tr>
<td>IgVH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mutated</td>
<td>40 (55)</td>
<td>33 (45)</td>
<td>0.015</td>
</tr>
<tr>
<td>unmutated</td>
<td>33 (45)</td>
<td>43.7</td>
<td></td>
</tr>
<tr>
<td>ZAP-70/IgVH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>positive/unmutated</td>
<td>21 (29)</td>
<td>20 (27)</td>
<td>0.007</td>
</tr>
<tr>
<td>discordant</td>
<td>48.3</td>
<td>42.9</td>
<td></td>
</tr>
<tr>
<td>negative/mutated</td>
<td>32 (44)</td>
<td>32 (44)</td>
<td>NR</td>
</tr>
<tr>
<td>CD38/IgVH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>positive/unmutated</td>
<td>17 (23)</td>
<td>24 (33)</td>
<td>0.0038</td>
</tr>
<tr>
<td>discordant</td>
<td>42.9</td>
<td>43.7</td>
<td></td>
</tr>
<tr>
<td>negative/mutated</td>
<td>32 (44)</td>
<td>32 (44)</td>
<td>NR</td>
</tr>
<tr>
<td>CD38/IgVH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>positive both</td>
<td>20 (27)</td>
<td>14 (19)</td>
<td>0.014</td>
</tr>
<tr>
<td>discordant</td>
<td>39</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>negative both</td>
<td>39 (53)</td>
<td>NR</td>
<td></td>
</tr>
</tbody>
</table>

NR: not reached.

Results. All 3 variables provided significant prognostic information with respect to the need of treatment. An intermediate prognostic group was identified for discordant cases. The predictive value of the three dual combinations is detailed in the table above. Concordant positive expression of CD38/ZAP-70 identifies an aggressive group of patients with the shorter time to first treatment (39 months versus not reached) and the lower percentage of discordant cases (19%). CD38/ZAP-70 positive expression predicted an unmutated status of IgVH gene in 75% of the cases whereas negativity for both antigens predicted a mutated status in 85% of cases. In the IgVH mutated group (n=40), 3 out of 11 patients treated were discordant cases who co expressed both antigens. Conclusion: in Binet A stage CLL patients, CD38/ZAP-70 positive expression allowed to identify a group of patients with a shorter time to first treatment (39 months) in concordant cases without the knowledge of the mutational IgVH gene status.

1280
LONG-TERM RESULTS OF THALIDOMIDE IN REFRACTORY AND RELAPSED MULTIPLE MYELOMA WITH EMPHASIS ON RESPONSE DURATION
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1Hospital Clinic, IDIBAPS, BARCELONA, Spain; 2Hospital Clinic, IDIBAPS, BARCELONA, Spain

Background. Thalidomide administered as a single agent produces a response rate of about 40% in patients with refractory or relapsed multiple myeloma (MM). However, the data on the duration of such responses is limited. Aim: to determine the...
quality and duration of responses to thalidomide in patients with refractory or relapsed MM. Patients and methods. Forty-two consecutive patients (22M:20F; median age: 61 years) with refractory (20) or relapsed (22) MM were given thalidomide as single agent at our institution from November 1999 to December 2008. Most of the patients (70%) had previously received 2 or more lines of therapy, and 56% had undergone autologous stem cell transplantation (ASCT). The drug was initially administered at a daily dose of 200 mg and later increased, depending on tolerance, by 100 to 200 mg every two weeks up to a daily dose of 800 mg. In responding patients, the dose of thalidomide was thereafter progressively tapered to a maintenance dose of 100 mg/day. No prophylactic anticoagulation was given. Results. Eighteen patients (43%) responded to thalidomide: 11 minimal responses (MR) and 7 partial responses (PR) according to the EBMT criteria. The median time to response was 3 months and the median duration of therapy in responding patients was 9 months. The reasons for discontinuing thalidomide in responding patients were: toxicity in 10 cases, progression in 4, and death due to pneumonia with respiratory failure in 2. In 2 additional patients treatment was stopped at the time when they were intensified with ASCT. The toxicity mainly consisted of peripheral neuropathy and fatigue. At the time of this analysis, all responding patients had progressed except one who remains in continued stable PR for more than 6 years after starting thalidomide therapy and for 3.5 years after thalidomide discontinuation. The median time to progression was 15.6 months (range: 1.3-70+), with a trend towards a longer duration for patients who achieved PR vs. MR (21.2 vs. 11.2 months, p=0.11). The median duration of response was 12.4 months (range: 0.3-67+) (17.2 months for PR vs. 9.7 months for MR, p=0.11). Conclusion. These results show that the effect of thalidomide in refractory/relapsed MM can be sustained, particularly in patients who achieve a good response, and support the investigation of this drug as maintenance therapy. 1281

THCY AND VASCULAR DISEASE. HOMOCYSTEINE AND VASCULAR DISEASE. IS THERE ANY RELATION AFTER ALL?

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General Hospital Hania Crete Greece, HANIA, Greece; Transfusion Service- General Hospital of Chania, KRETE, GRECE

During the last years, after the completion of several epidemic studies, it is supported by many researchers that homocysteine is another risk factor of vascular disease. The aim of our study was to evaluate the homocysteine’s plasma levels in patients who suffered from coronary disease or a cerebrovascular accident. The patients were categorized into 3 groups. Group A: 130 healthy volunteer blood donors, of whom 108 were men with mean average age 35.7 years old (19-57) and 22 women with mean average age 33.95 years old (22-55). Group B: 68 with cerebrovascular accident, confirmed with brain CT or MRI, of whom 31 were men with mean average age 52.6 years old (31-75) and 37 women with mean average age 51.21 years old (35-67). Group C: 54 patients with an acute coronary syndrome, of whom 26 were men with mean average age 50.5 years old (35-61) and 8 women with mean average age 44.6 years old (34-55). In groups B,C patients with other risk factors, such as hypertension, diabetes mellitus, hyperlipidemia etc, were not included. The homocysteine measurement was performed with Elisa (ABBOT-AXSYM) and the blood test was done, in all groups, under the same circumstances. In the following table see the results.

<table>
<thead>
<tr>
<th>Group</th>
<th>Men 11.4±5.8µmol/L</th>
<th>Men 11.3±2.94µmol/L</th>
<th>Men 11.2±2.4µmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women</td>
<td>8.98±2.13</td>
<td>8.81±2.52</td>
<td>14.1±7.5</td>
</tr>
</tbody>
</table>

If we exclude those women with cardiovascular disease who had higher homocysteine’s plasma levels from women of the other groups, significant differences among the levels of the others were not noticed. Thus many questions remain to be answered before THcy is finally considered as a risk factor of cardiovascular disease or stroke. Many more studies are needed before we are able to focus our attention in therapy and prevention.

1282

ANALYSIS OF THE EFFECTIVE AGENTS IN DEVELOPMENT OF FACTOR INHIBITOR IN HEMOPHILIC PATIENTS EVALUATION OF 445 PATIENTS IN CENTRAL PART OF IRAN

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Isfahan University of Medical Sciences, ISFAHAN, Iran

Backgrounds. In hemophilia the development of inhibitors is a serious problem. Inhibitors reduce the efficacy of haemostatic treatment and clearly cause additional morbidity. Understanding the effective agents in arising factor inhibitors could be helpful in management of hemophilic patients. Aim: To evaluate the development of inhibitors and analysis the effective agents in it, in patients with hemophilia. Method: A comprehensive study undertaken in Hematology-Oncology Department- Isfahan University of Medical Sciences. All hemophilic patients (445 patients) underwent frequent testing for inhibitors and the development of an inhibitor was defined by a titer > 0.5 Bethesda units (BU)/ml in any sample. Clinical history, Laboratory and treatment data of all patients were studied in January 2006. Results: from 401 men and 44 women with factor deficiency with Mean±SD age of 23.25±13.15, 27 patients (6.06%) showed factor inhibitor. The mean duration between diagnosis of disease and inhibitor arising was 15.26 years. From them 26 had Factor (F) VIII deficiency who were 7.6% of patients with FVIII deficiency, and one had FIX deficiency. According to forward stepwise logistic model (with percentage correct of 95%) treatment with factor concentrates with 2.9 times, and FVIII deficiency with 12.7 times chance correlate with factor inhibition. Other agents like, severity of disease, blood group and age of patients do not enter in the model. In this study 12.4% of patients who used factor concentrates, develop factor inhibitor (0.000). Conclusion: These data provide estimates of the rate of inhibitor in factor deficiencies. Beside several advantages of factor usage in treatments of hemophilic patients, the more chance of coloration between inhibitor development and it should be noticed.
The collection of red blood cells (RBC) and platelets (plt) by multicomponent collection (MCC) is a mean to reduce donor exposure of polytransfused patients. We compared plt activation and plt function parameters in platelet concentrates (PC) collected by two different devices during 7 days storage to estimate a possible association between the level of activation and collection modality as well as maintained platelet function. Materials and Methods. Fifteen donors, each with two donations, were included in our study. For each donor we used the TRIMA Access (Gambro BCT) and AMICUS (Baxter) with an interval of at least two months in between. PC were stored under agitation (Gambro BCT) (T-PC) and AMICUS (Baxter) (A-PC) with an interval of at least two months in between. PC were stored under agitation (Gambro BCT) (T-PC) and AMICUS (Baxter) (A-PC) with an interval of at least two months in between.

**Introduction**

The collection of red blood cells (RBC) and platelets (plt) by multicomponent collection (MCC) is a mean to reduce donor exposure of polytransfused patients. We compared plt activation and plt function parameters in platelet concentrates (PC) collected by two different devices during 7 days storage to estimate a possible association between the level of activation and collection modality as well as maintained platelet function. **Materials and Methods.** Fifteen donors, each with two donations, were included in our study. For each donor we used the TRIMA Access (Gambro BCT) (T-PC) and AMICUS (Baxter) (A-PC) with an interval of at least two months in between. PC were stored under agitation (Gambro BCT) (T-PC) and AMICUS (Baxter) (A-PC) with an interval of at least two months in between.

**Bacterial contamination of apheresis product for autologous peripheral blood transplantation and their correlation with post-transplant behaviour**


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Peripheral blood progenitors cells (PBPCs) for autologous transplantation require a careful manipulation in several steps. Bacterial contamination is a well known risk in most of the transplant units, although their clinical significance remains controversial. We reviewed the clinical records of our transplanted patients. The aim was to know the incidence of bacterial contamination in PBPCs, and how this affects post-transplant infections, time to engraftment, transfusion requirements, days of fever and hospitalization of our patients. A total of 134 transplanted patients received 525 aliquots of PBPCs product, a median of 3 (1-20) per patient. 127 patients (95%) had fever in the post-transplant period; 117 (92%) of them had positive blood cultures obtained from peripheral vein or central venous catheter. The most frequent bacteria were: coagulase-negative Staphylococcus in 53% patients, followed by coagulase-positive Staphylococcus in 5%, E.coli in 3%, Streptococci in 3%, and others in less than 1%. Bacterial contaminated PBPCs were infu ed to 11 patients. The bacteria isolated from these aliquots were: 2 Streptococcus viridans, 5 coagulase-negative Staphylococcus, 2 Corynebacterium and 2 no identified Gram-positive bacillus. These patients received no prophylactic antibiotic therapy, but at the moment of infusion peripheral blood granulocyte count were normal. In three out of these eleven patients receiving contaminated PBPCs, the same bacteria was isolated in blood. No difference was found between patients receiving grafts with and without contaminated PBPCs in terms of days of fever (6-1-7) vs (4-1-23), transfusion requirements, days of hospitalization, days of engraftment of granulocytes (12(10-12) vs 11(9-25)) and platelets (15(10-25) vs 12(6-35)). In the group of patients receiving contaminated PBPCs, no difference was found between the three patients with the same bacteria and the eight with a different one it in terms of: days of hospitalization, days of fever 4(1-6) vs 3(2-7) respectively, day of granulocytes 12(10-12) vs 12(10-15) and platelets engraftment 11(10-15) vs 13(12-21) respectively. From our experience, it seems that the infusion of contaminated hematopoietic cells has not clinical translation although there are few cases. The microorganism most frequently isolated in the contaminated PBPCs aliquots and in the blood cultures of patients with fever was S. epidermidis. As this bacterium is frequently associated with vascular catheter infections, we cannot know if the contamination is due to infused product or not.

**Therapeutic cytapheresis: an adapted strategy for leukodepletion**

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**Background and Aims.** Hyperleukocytosis may represent a life-threatening condition for patients with ALL, AML, CLL or CML. Elevated white blood cells counts may cause acute respiratory distress syndrome, intracranial bleeding and the tumor lysis syndrome after chemotherapy. Therapeutic leukapheresis dramatically decreases the number of circulating leukocytes with beneficial effects on hyperviscosity and leukostasis.
sion. Moreover leukocytoreduction prior to chemotherapy can reduce metabolic and renal complications due to rapid cellular lysis. Despite benefits, leukapheresis may pose multiple concerns because of patient’s poor clinical conditions like concomitant anaemia, thrombocytopenia and hypertension. ASFA Committee classifies hyperleukocytosis as category I indication for therapeutic apheresis, nevertheless the effect is only temporary and the institution of appropriate chemotherapy is essential. We report the experience of performing leukocytoreduction at our Apheresis Service employing a third generation cell separator adapted on the basis of target cells separation characteristics. Methods. A summary of the patients characteristics is given in Table 1.

Table 1. Characteristics of the patients (n=16).

<table>
<thead>
<tr>
<th>Male/female</th>
<th>Age (years)*</th>
<th>Diagnosis:</th>
</tr>
</thead>
<tbody>
<tr>
<td>10/6</td>
<td>56 (33-85)</td>
<td>CLL</td>
</tr>
<tr>
<td></td>
<td>6 (37%)</td>
<td>ALL</td>
</tr>
<tr>
<td></td>
<td>5 (32%)</td>
<td>CML</td>
</tr>
<tr>
<td></td>
<td>3 (19%)</td>
<td>NKL with circulating blasts</td>
</tr>
<tr>
<td></td>
<td>1 (6%)</td>
<td>AL in MDS 1</td>
</tr>
</tbody>
</table>

Vital parameters:

- **Peripheral blood values:**
  - Pre-apheresis
  - Post-apheresis
- **WBC (x10^9/microL)**: 251.2 (87.75-514.0) / 115.5 (46.6-266.0)
- **Hb (g/dL)**: 8.6 (4.0-11.3) / 6.6 (3.7-9.6)
- **PLT (x10^3 /microL)**: 75 (6-216) / 43 (6-99)

- **Mean value and range in brackets.**

All patients signed a detailed informed consent. An immediate pre-apheresis blood count was carried out for every procedure; moreover, blood cell morphology was assessed by peripheral blood smear and May Grunwald-Giemsa staining. Leukocytes were removed by continuous flow centrifugation leukapheresis (COBE Spectra® device, Lakewood, CO, USA) utilizing citrate dextrose solution A as anticoagulant. The mononuclear cells (MNC) collection program was used in all cases, switching into manual mode and appropriately modifying the separation parameters. The separation factor of the device varied from 500 to 1000 depending on the target cells size (small lymphocytes or large blasts). The collect pump rate was always set at 5.0 mL/min. Patients were carefully monitored as respect to vital signs (blood pressure, heart rate, oxygen saturation). Calcium gluconate was infused i.v. continuously to prevent or minimize citrate toxicity. Isovolemia was maintained by carefully replacing the withdrawn volume with 5% human serum albumin in 0.9% NaCl solution i.v. continuously. In case of platelet count less than 20x10^9/µL, prompt transfusion was administered before and/or after leukapheresis; red blood cell transfusion when necessary was delayed to completion of apheresis to avoid further increase in hyperviscosity. Results. From 2001 we have performed 34 apheresis procedures in 16 patients, whose characteristics are detailed in table 1 and 2. We obtained a decrease in circulating leukocytes (to less than 100 x10^9/µL) by a unique leukapheresis in 7 patients (44%) and by 2 procedures in 9 (56%). No significant adverse effects occurred. Conclusions. In our hands, the strategy based on the MNC program adapting the leukocytes morphology showed to be effective as well as highly tolerated and safe. This variant provided efficient leukocytes reduction; 5% Albumin administration was able to preserve from the risks of hypotension even critically ill patients.

**Autoimmune Hemolytic Anemia After Allogeneic Stem Cell Transplantation**

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The development of autoimmune hemolytic anemia (AIHA) is one of the adverse effects of allogeneic bone marrow transplantation. AIHA is thought to be due to antibodies produced by the donor’s immune system against antigens on RBC’s of donor origin. Measure the incidence of AIHA after allogeneic stem cell transplantation. The records of 213 patients (male=151, female=62) transplanted for hematologic and transplant malignancies, referred to our immunohematology laboratory between Jan 2000 and Jan 2006, were reviewed. Patients who experienced hemolysis with a positive DAT were selected for further study. The presence of anticytochrome autoantibody was analyzed on the red blood cells and in the serum of patients. All cases were studied using polyvalent and monospecific antisera (ID-Diamed) as well as IgG subclasses. Eluates were performed with acid elution test. A diagnosis of AIHA was made in 5 out of 213 patients (2.36%) referred to our laboratory. The median age was 24 years (range, 16-47 years); 4 of them were men. Two had 1- and 8- ALL, one B-CLL, one NHL with circulating blasts 1 (6%); 3 patients had warm-type AIHA and 2 mixed (warm and cold) AIHA. Warm-type AIHA had an earlier onset beginning 4-7 months post-transplant, whereas mixed-type developed 12-13 months post-transplant. Selected and irradiated red cells were given to 3 patients with severe and symptomatic anemia, while the initial treatment consisted of steroids. Both patients with mixed-type AIHA are in complete remission. One patient with warm-type has compensated hemolysis without treatment. One patient died due to resistant underlying disease and the last patient failed to respond to steroids, presented graft failure and received a second transplant. AIHA following bone marrow transplantation is a rare side effect. Its frequency ranged to 2.5% and there is a male predominance. The serologic findings are the same as in AIHA not associated with transplantation. The hemolysis may be severe and chronic. There is considerable variation in prognosis, reflecting the treatment modalities used.

**Therapeutic Plasma Exchange for Thrombotic Microangiopathy After Hematopoietic Stem Cell Transplantation: A Single Centre Experience**

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Thrombotic microangiopathy (TMA) after hematopoietic stem cell transplantation (HSCT) is an uncommon but serious complication. The etiology is still unclear although endothelial damage is a common mechanism. No effective treatment is available, although the therapeutic plasma exchange (TPE) with fresh frozen plasma (FFP) could partially improve its manifestations. The aim of this study was to retrospectively analyse TMA cases in our Centre and their response to TPE. In the last 10 years, 389 patients underwent allogeneic HSCT, 10 of them (2.6%) developed TMA and initiated plasma treatment. These patients, 5 men and 5 women, with a median age of 50.5 years (range 4-69) were diagnosed acute myelodysplasia (n=4), acute lymphoblastic leukemia (n=2), Paroxysmal Nocturnal Hemoglobinuria (n=2), Non-Hodgkin’s Lymphoma (n=1) and Chronic Myeloproliferative Syndrome (1). It was the first allogeneic HSCT for 6 patients, the second for 3 and the third for 1 of them. The conditioning regimen was myeloablative in 5 patients and of the separation parameters was peripheral blood from HLA-identical related donor for 7 patients and the other 3 received bone marrow (1 HLA- identical related, 1 mismatched related, 1 identical unrelated). Cyclosporine (CSA) was used for...
global QoL of patients with multiple myeloma after the HSCT is lower with increasing age in both groups patients and with nicotinism in 0.01).

Patients and Methods. The total number of respondents after the transplantation from 2001 to 2003 was 80 and the return rate of questionnaires was 70% (56 respondents: 32 respondents (15 men, 14 women) with multiple myeloma and 24 respondents (11 men, 15 women) with malignant lymphoma). All respondents with multiple myeloma were after the autologous HSCT. 22 respondents with malignant lymphoma were after allogeneic HSCT. The average age of 2.5 L (1.4-3.9) of FFP with a fluids change of 100% remaining iso-

Backgrounds. The cross-sectional and retrospective study analyses the selected factors which influence global quality of life (QoL) of patients with multiple myeloma and malignant lymphoma after the hematopoietic stem cell transplantation (HSCT). Aims. 1. to verify the applicability of the Czech version of an international generic European Quality of Life Questionnaire - Version EQ-5D for the evaluation of global QoL of patients after the HSCT at the Department of Clinical Hematology of the 2nd Internal Clinic in the University Hospital and Medical Faculty of Charles University in Hradec Kralove, Czech Republic. 2. to evaluate the global QoL of patients with multiple myeloma and malignant lymphoma after the autologous HSCT. Methods. 1. The study was conducted in the Department of Clinical Hematology of the 2nd Internal Clinic in the University Hospital and Medical Faculty of Charles University in Hradec Kralove, Czech Republic. 3. to analyse factors which influence global QoL of patients with multiple myeloma and malignant lymphoma after the HSCT at the Department of Clinical Hematology of the 2nd Internal Clinic in the University Hospital and Medical Faculty of Charles University in Hradec Kralove, Czech Republic, 3. to analyse factors which influence global QoL of patients with multiple myeloma and malignant lymphoma after the HSCT at the Department of Clinical Hematology of the 2nd Internal Clinic in the University Hospital and Medical Faculty of Charles University in Hradec Kralove, Czech Republic. 3. to analyse factors which influence global QoL of patients with multiple myeloma and malignant lymphoma after the HSCT at the Department of Clinical Hematology of the 2nd Internal Clinic in the University Hospital and Medical Faculty of Charles University in Hradec Kralove, Czech Republic.

Backgrounds. Pacing with having been the object of studies for long time before the instruments for assessment in specific illnesses, like haemophilia, were constructed. Some research has already indicated that individual sub-scales in Cope Strategies Questionnaires (CSQ) are more meaningful than composites (Jensen MP et al., 1992). Aims. The aim of this study is to describe pain coping strategies among patients with haemophilia and find out if the results are related to the severity of disease. Methods. A group of 24 adult patients with moderate and severe haemophilia is presented. The patients’ coping with pain is assessed using the Pain CSQ Adapted for Haemophilia (PCSQ, Barry and Elander, 2002). This questionnaire was translated into Serbian, according to the recurred guidelines. Clinical severity of disease is measured using the frequency of bleeding episodes in the previous year (Solovieva S, 2001). Physical activity level is measured on a two-point scale. Statistical analysis, firstly performed, was based on the three originally defined factors in the PCSQ: negative thoughts about pain, coping attempts and passive adherence. Afterwords, it was based on 14 subscales, each one grounded on of 3 to 6 items. Results. In the factor analysis, no differences are found in coping with pain between the groups with clinically severe and moderate disease (p>0,05), between patients with biologically severe and moderate haemophilia (p>0,05) and between those with difficulties in hard (moderate) physical activity and those with difficulties in any (no) activity (p>0,05). When using sub-scale scores, differences in pain coping strategies were found between the groups. Patients with difficulties in hard or moderate physical activities ignored pain sensations and increased behavioural activities, using them like preferred strategies more than people with difficulties in any or no activity (p<0,05). On the other hand, patients with difficulties in any activity used clotting factor and ice more often to cope with pain (p<0,05). Patients with clinically moderate disease also ignored pain sensations more willingly than those with severe haemophilia (p<0,026), who relied on praying and hoping (p<0,01) and used anger self-statements more when in pain (p=0,054). Patients with biologically moderate disease used ice more than those with severe disease (p=0,059). Summary. The results based on factor analysis suggest that the severity of haemophilia may not be the factor determining the type of patient’s pain coping strategy. The results based on sub-scales analysis suggest that possibly it would be better to analyze scores from the questionnaire in this way, rather than putting sub-scales together into three factors.
ti c Index we examined fatigue severity and fatigue interference within patient groups stratified by IPI. Results. Fatigue was reported by 77.3% of NHL patients predominantly in those with aggressive lymphoma (95%). Almost two thirds (60.5%) of patients experienced fatigue at the moderate-to-severe level. Aggressive NHL patients reported significantly more fatigue interference with patients’ daily lives than indolent NHL patients: mean RIFI interference score was 9.9% (SD = 2.50) vs 2.16 (SD = 2.55) (p<0.05). The distribution of patients according to the IPI was as follows - IPI-1: IPI-2: IPI-3: IPI-4: IPI-5 - 5.3: 5.3: 19.3: 70.1 (%) for aggressive NHL and 40.0: 30.0: 20.9: 8.1 (%) for indolent NHL. Fatigue severity differed significantly in the IPI groups (p<0.001). Patients at low risk according to the IPI both in aggressive and indolent NHL groups had no fatigue. Patients at low-intermediate risk IPI experienced mild fatigue (mean 5.2, SD=2.3 for aggressive NHL; mean 2.1, SD=1.8 for indolent NHL). IPI-3 group was characterized by moderate fatigue (mean 5.7, SD=1.5 for aggressive NHL; mean 4.1, SD=2.4 for indolent NHL). Patients at high risk IPI had severe fatigue (mean 7.5, SD=1.4 for aggressive NHL; mean 7.1, SD=0.9 for indolent NHL). Significant differences in fatigue interference with patients’ daily lives were found across IPI groups (p<0.001). Conclusion. Our results show that fatigue is a prevalent and distressful symptom in new NHL patients. It is much more pronounced in patients with aggressive lymphoma. Furthermore, we found that a certain IPI group is strongly distinguished by fatigue severity and its impact on quality of life. The findings support the suggestion that fatigue should be discussed as an important prognostic factor in this patient population.

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USAGE OF EICOSAPENTAENOIC ACID AND HIGH PROTEIN CONTAINING ENTERIC FEEDING PRODUCT IN MALIGNANCY RELATED WEIGHT LOSS OF CHILDREN

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The aims of nutrition therapy are preventing weight loss and improving functional capacity and life quality in cancer patients. Clinic effectiveness of standard nutritional support is limited in patients with tumor related weight loss. We aimed to observe weight changes of actively chemotherapy receiving patients by using eicosapentaenoic acid and high protein containing enteral nutrition product (ProSure®). 46 patients (27 (58.7%) male, 19 (41.3%) female) were included to study. Mean age of patients was 80.5±41.38 months (20-187 months). 39 patients had diagnosis of leukemia, 7 had that of a solid tumor. All patients were receiving chemotherapy actively. Basal weight and body size of patients were recorded and they were suggested to use enteral products twice a day (morning - evening) in addition to their normal feeding. Patients were followed with regular intervals and their data were recorded. Their tolerance and regular use of the product were questioned. Patients were followed approximately 92±40.6 days. 33 (71.7%) patients had consumed and tolerated the product, 15 (28.3%) patients had consumed less than the suggested amount because of its taste. Body weights of 20 (45.5%) patients were increased while that of 9 (19.6%) patients were decreased. No significant weight changes were observed in 17 (37%) patients. In conclusion, body weights of 80.4% of our patients were preserved, and that of 43.5% were increased.

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SATISFACTION WITH IRON CHELATION THERAPY IN PATIENTS WITH THALASSEMIA, SICKLE CELL DISEASE, AND MYELODYSPLASTIC SYNDROMES

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Backgrounds. Thalassemia, sickle cell disease (SCD), and myelodysplastic syndrome (MDS) patients require regular blood transfusions as part of their treatment programme. Regular blood transfusions can lead to iron overload, which if untreated, IQ will result in morbidity and mortality. Removal of excess iron with currently available iron chelation therapy (ICT) requires 8-12 hour infusions, repeated 5-7 days per week. This time consuming therapy is burdensome to patients and may impact on satisfaction with ICT. Aims. To assess satisfaction with ICT and factors associated with satisfaction in patients with thalassemia, SCD, or MDS. Methods. A 28 item satisfaction questionnaire was developed and is currently being validated. The instrument comprises four domains that assessed satisfaction with ICT (acceptability; burden; perceived effectiveness; and side effects) and was administered at a single time point to 110 patients currently receiving ICT in the US (thalassemia: n=41, SCD: n=9) or UK (thalassemia: n=40, SCD: n=14 MDS: n=6). Simple regression analyses were then conducted to explore factors explaining satisfaction with ICT. Results. The mean age was 30.87 years (SD=14.95), with 63 females and 47 males. A total of 54% responded that they found their ICT inconvenient or very inconvenient compared to 26% who stated that they found their therapy convenient or very convenient. Further, 22% reported they were either very satisfied or satisfied with their prior ICT compared to 31% of patients stated that they were either dissatisfied or very dissatisfied. When asked overall, how did the side effects of chelation therapy meet your expectations, 22% stated that they were either much better or somewhat better than their expectations, with 52% stating that they were either somewhat worse or much worse than their expectations. Simple regression analyses revealed that whether patients experience side effects (R²=15%, p<0.0001), and the number of doses per week (R²=7%, p<0.001) were positively related to acceptability of ICT, whereas the number of doses missed in the last 7 days was negatively linked (R²=6%, p=0.01). Whether patients experienced any side effects were also significantly and negatively associated with satisfaction with side effects (R²=9%, p=0.001). Disease type (R²=39%, p<0.0001) and unemployment (R²=31%, p<0.001) were positively related to satisfaction with ICT. Whether side effects were experienced was also significantly and negatively associated with satisfaction with side effects (R²=31%, p<0.0001). Summary/Conclusions. Results indicated that the majority of patients found their ICT inconvenient. One third of patients stated that they were dissatisfied with their ICT. Further, satisfaction is significantly influenced by a number of important factors related directly to ICT. Whether patients experience any side effects appears to be the single most important determinant of satisfaction and was associated with three of the four satisfaction domains: acceptability of ICT; burden of ICT and satisfaction with side effects.

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DOWN-REGULATION OF OSTEOCALCIN BY IMATINIB-INDUCED INHIBITION OF CELL PROLIFERATION

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Backgrounds. Activated stem cells from haematological malignant tumours may show also characteristics of mesenchymal stem cells. In c-kit (CD117) positive cells, at least two splice variants of osteocalcin (OCN) were described. Aims. Aim of our study was to elucidate whether the expression of OCN and its splice variants relate to differentiation stages of of haematopoietic cells (HL60) and an associated regulation of the transcription factors AML1 and AML3. Additionally, we wanted to clarify how cell-differentiating agents like vitamin D3 and imatinib (Glivec) affect proliferation of cells and expression of the genes named above. Methods. After incubation with differentiating agents (vitamin D3, imatinib), mRNA-expression of OCN, the transcription factors AML1 and AML3 and various metabolic genes were quantified by means of RT-PCR. Results. Our RT-PCR quantifications showed that after addition of vitamin D3 and imatinib, OCN appeared to be down-regulated. Alike observed at all marker genes for metabolism and haematoepoiesis, the effect of inhibition of AML1 and AML3 was strongest with vitamin D3. After imatinib treatment, in all cell-lined analysed, the dose-dependent repression of proliferation is coupled with inhibition of OCN- and AML1-mRNA-expression. As opposed to the down-regulation of markers for immature cells, the differentiation marker Lox (lysyloxidase) was stimulated. Conclusion: In the cell-lines observed, differentiation leads to a decrease of the expression of OCN and the associated transcription factors. Further studies shall prove the effect of differentiation agents on healthy cells.
Cryopreservation of Peripheral Blood Progenitors for Autologous Transplantation in Hematological Malignancies with Different Concentration of Cryoprotectant - A Five Year Center Experience

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In this study we present our five year center experience with cryopreservation of PBSC and autologous transplantation in 42 patients with hematological malignancies treated in a period 2000-2005 at Department of Hematology, Skopje. Material and Methods. diagnosis of patients were (9 AML, 11 NHL, 10 MM, 12 HD) and median age transplant was 44 years (7-63). Mobilization of PBSC was provided with Etoposide (VP-16)+ G-CSF 10mcg/kg in AML patients, and high dose Cyclophosphamide 4-5gr/m²+G-CSF 10 mcg/kg or alone G-CSF 10 mcg/kg in patients with lymphoproliferative diseases. Collected PBSC were cryopreserved in solutions with 5% DMSO in 20 patients and 10% DMSO in 22 patients, computer programmed until -80°C, and stored different period in liqueur nitrogen on -196°C. Autologous transplant was performed with conditioning consisted of myeloablative high-dose chemotherapy, BuCy in AML patients, high dose Mel in MM patients, BEAM and if the patient is in remission, BEAM and ICE in NHL patients and BEAM in HD patients. Cell viability was assessed by fluorescence microscopy using acidine orange dye exclusion. Results. A total of 103 PBSC cryopreservation procedures were performed in our group of patients with median 3 (2-5) apheresis procedures. Median period from storage of cryopreserved PBSC grafts until thawing was 46 days (52-60). Total number of infused CD34+ cells was between 2.0-15×10^6/kg and median number of mononuclear cells was 4.2×10^6/kg (1.7-7.2). The amount of infused DMSO solution ranged between 210-650ml (median 480 ml) with DMSO concentration ranging 23 ml-50 ml (median 35 ml) in a group preserved with 10% DMSO and 18-28 ml (median 19 ml) in 5% DMSO cryopreserved grafts. The viability of the fresh harvests before storage was median 97% (range 68, 5-99, 9%). The poorer viability was associated with harvest cell count. Bellow 300×10^6/L the median viability was 98% and only 2/42 cases had <85% viable cells. Harvests count above 300×10^6/L the median viability was 78% (67, 8-99%). In a group of patients that received PBSC grafts preserved with 10% DMSO, also revealed signs of mild DMSO infusion related toxicity (22% vs 14%). Hematopoietic recovery was similar in both groups, for Ne>0.5×10^9/L on day +9 (8-10), Pt >2.0×10^9/L on day +12 (11-14). Our results confirm that the infusion of cryopreserved autologous PBSC in hematological malignancies revealed successful engraftment in all patients and good cell viability. We did not registered hard to mobilize patients and graft failure.

No Significant Increase of Circulating CD34+ Stem Cells in Patients After Ischemic Stroke

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Backgrounds. Stroke has a great socio-economic impact. Despite this the therapeutic influence on outcome is limited. There is an increasing suggestion that hematopoetic stem cells (HPC) may be able to induce repair processes after an ischemic event. Aim: Our objective was to determine whether patients with an ischemic cerebral event have elevated levels of circulating HPC. Material and Methods. diagnosis of patients were 21 (20 ischemic stroke and 1 intracerebral hemorrhage). Isolated CD34+ cells were counted and then cultured with FACSort according to the ISSAGE protocol using a dual platform method. The total leukocyte and granulocyte count was evaluated by a Coulter MDII analyzer. Results. The 10 healthy blood donors had a mean leukocyte count of 6.1±1.4×10^9/L with 0.033±0.028% CD34+ cells and a CD34+ cell count of 1.85±1.45×10^6/µL. Mean age of the 28 stroke patients (10 females/18 males) was 67.7±12 years. 26 of the patients had an ischemia of the MCA, 2 of the ACA, 1 of the PCA and 1 of the PICA. They showed a mean leuko-cyte count of 6.9±2.3×10^9/L with 0.027±0.016% CD34+ cells and 1.66±1.08×10^6/µL CD34+ cells at time of admission. Measurement showed a mean leukocyte count of 8.1±3.7×10^9/L with 0.018±0.022% CD34+ cells and 1.51±1.08×10^6/µL CD34+ cells 24 hours after admission. 3 days after admission measurement showed a mean leukocyte count of 7.5±3.1×10^9/L with 0.021±0.022% CD34+ cells and 1.33±0.76×10^6/µL CD34+ cells and 7 days after admission a mean leukocyte count of 7.1±1.8×10^9/L with 0.027±0.050% CD34+ cells and 2.03±1.10×10^6/µL CD34+ cells. There was no significant difference in leukocyte count or circulating CD34+ cell count between stroke patients and the healthy control group. 24 hrs after admission patient leukocyte counts peaked potentially explained by an acute phase reaction. However absolute CD34+ cell counts of the complete patient group and of so far analysed subgroups of cortical, subcortical and territorial stroke remained without a statistically significant change. But subgroup analysis between cortical, subcortical and territorial stroke patients as well as time from stroke to admission seems to show a trend for correlation with CD34+ cell number, leucocyte counts and granulocyte counts. Summary/Conclusions. We found no evidence of a general increase of circulating CD34+ stem cells in stroke patients that would indicate an involvement in a postulated repair process. But a subgroup analysis of a larger patient group is necessary to elucidate a possible association between circulating CD34+ cells and stroke. However, the possibility that CD34+ cells home to the site of tissue damage without a measurable increase of circulating CD34+ cells still remains.

Enhanced Engraftment of Human Umbilical Cord Blood Derived CD34+ Stem Cells in BALB/c Mice by Cotransplantation of Mesenchymal Stem Cells

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Backgrounds. Umbilical cord blood (UCB) is considered as an attractive alternative source of hematopoietic stem cells for allogeneic stem cell transplantations. However the rate of UCB CD34+ stem cells graft is low. Mesenchymal stem cells (MSC) have been implicated in playing an important role in hematopoetic stem cell engraftment. Aim: In this study we examined the effect of human umbilical cord blood (UCB)-derived CD34+ cells in irradiated Balb/C mice. Methods. Human UCB CD34+ cells were obtained from full-term normal deliveries. Isolated CD34+ cells were counted and then cultured in Stemline II Hematopoietic stem cell expansion medium supplemented with 100 ng/ml SCF, and 100ng/ml TPO in 24-well plates. The cultures were incubated at 37°C in a fully humidified atmosphere with 5% CO2, and maintained over two weeks and half the medium was exchanged twice a week. Viability test was performed by trypan blue staining (100%). The direct determination of the absolute count of CD34+ was assessed by Flow cytometry (90%). Irradiated (7 Gy) Balb/C mice (n=12) were transplanted intravenously with 0.2 to 1.0×10^6 UCB CD34+ cells in the presence or absence of 0.25 to 1×10^6 culture-expanded human bone marrow-derived MSC. The mice in every group on day 11 after transplantation were killed and their spleen dissected. In every group colony assay were performed. For assessing the presence of stem cells in colony, UCB CD34+ cells labeled with super paramagnetic iron oxide (SPIO) were transplanted. After establishing the presence of colonies in spleen, Prussian blue staining was performed. Results. Cotransplantation of low doses of UCB CD34+ cells (0.2 and 0.3×10^6) and MSC (0.5 and 1×10^6) resulted in a four-fold to five-fold increase in colony forming unit spleen, in comparison with engraftment of UCB CD34+ stem cells without MSC after 11 days. After Prussian blue staining Fe+2 granules were observed. This indicates these cells in the colony were UCB CD34+ stem cells that were engrafted. Conclusions. The results showed that cotransplantation of MSC with UCB CD34+ cells; promote engraftment of UCB CD34+ cells.

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EARLY HEMATOPOIETIC RECOVERY AFTER AUTOLOGOUS PERIPHERAL BLOOD STEM CELL (PBSC) TRANSPLANTATION IN RELATION TO THE NATURE OF THE INFUSED PRODUCT: UNSELECTED VERSUS SELECTED PBSC


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In the setting of autologous stem cell transplantation, purified CD34+ cell selection by immunomagnetic beads removes tumor cells from PBSC apheresis product, diminishing the relapse rate and outcome. Some authors have reported that this purging may delay neutrophil and platelet count recoveries after transplantation. To analyze differences in early hematopoietic recovery (day in reaching neutrophil count > 0.5×10⁹/ L and platelet count > 20×10⁹/ L) after autologous PBSC transplantation, when selected versus unselected products are compared. We studied 160 consecutive PBSC transplantations (79 with unselected and 81 with selected PBSC), which were performed in our center over the last ten years. There were not statistically significant differences between both groups of patients in terms of infused cellularity: Unselected PBSC 3.86×10⁹/kg and selected PBSC 3.35×10⁹/kg (p=0.308). All patients received G-CSF daily (300 µg, subcutaneous) from day 1 until the Neutrophils count > 500/µm³, for 3 consecutive days or Neutrophils count > 1000/µm³. We did not find differences between the two groups in the day in which neutrophil early graft took place (unselected PBSC product, day +11,11; selected PBSC product, day +11,31 (p=0.104). Similarly, platelet recovery was not significantly different (unselected PBSC product, day +12,77; selected PBSC product, day +13,09 (p=0.101). When analysis was performed based upon the infused cellularity, we found the following Results: 1) CD34+ cells infused < 2×10⁶/kg: unselected PBSC product, day +12,17 for neutrophils recovery, vs day +12,50 for selected PBSC product (p=0.747) and unselected PBSC product, day +18 for platelets recovery vs day +19,83 for selected PBSC product (p=0.857), 2) CD34+ cells infused 2-4×10⁶/kg: unselected PBSC product, day +11,12 for neutrophils recovery, vs day +11,57 for selected PBSC product (p=0.210) and unselected PBSC product, day +12,46 for platelets recovery vs day +13,11 for selected PBSC product (p=0.159). 3) CD34+ cells infused > 4×10⁶/kg: unselected PBSC product, day +10,40 for neutrophils recovery, vs day +10,83 for selected PBSC product (p=0.757) and unselected PBSC product, day +11,87 for platelets recovery vs day +12,48 for selected PBSC product (p=0.287). In our study, we demonstrate that, although there is a tendency to a more delayed early graft for selected PBSC products, there were not statistically significant differences between selected and unselected autologous PBSC transplantations in terms of early hematopoietic recovery. This setting did not substantially vary when infused cellularity in each group was compared.

1300

EVALUATION OF MESENCHYMAL STEM CELL EFFECT ON HOMING OF UMBILICAL CORD BLOOD STEM CELL IN BALB/c MOUSE WITH CLINICAL 1.5-T MRI

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Backgrounds. MSCs have been implicated as playing an important role in hematopoietic stem cell engraftment in co-transplantation experiments. We evaluated the effect of these cells in the homing of umbilical cord blood isolated CD34+ cells in irradiated and cyclosporine treated mice. In this study, we used murine clinical 1.5-T MRI as a tool for tracking transplanted hUCB CD34+ cells in animal models and to evaluate the effect of MSCs in homing of these cells. Methods. Culture-expanded bone marrow derived MSCs were characterized by immune phenotyping and cultured under conditions promoting differentiation to osteoblasts or adipocytes. Culture-expanded umbilical cord blood derived CD34+ cells were labeled with iron-oxide nanoparticles (Endorem™). Irradiated (7.5 Gy) and cyclosporine treated Balb/c mice were transplanted intravenously with labeled UCB CD34+ cells in the presence or absence of culture-expanded MSCs. Mice underwent MR imaging using 1.5-T MRI equipment, before and after intravenous injection of hUCB CD34+ cells labeled with SPION through simple incubation with protamin sulfate. Results. After injection of iron oxide-labeled hematopoietic cells, a significant decrease in MR signal intensity was observed in the bone marrow. The signal intensity reduction in bone marrow was significantly stronger after co-transplantation with MSCs, compared to transplantation of UCB CD34+ cells alone. Histochimical examination for iron by the Prussian Blue Method in spleen colony forming units, confirmed these results. Conclusion: Co-transplantation of hMSCs with UCB CD34+ cells enhances their engraftment. This can be detected and evaluated in vivo with clinical 1.5-T MRI.

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HEPATIC VENO-OCCULSIVE DISEASE: A SINGLE CENTER EXPERIENCE WITH DEFILOBRIDE

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Backgrounds. Veno-occlusive disease (VOD) of the liver, renamed as sinusoidal obstruction syndrome is a common and possibly fatal complication of hematopoietic stem cell transplantation. The incidence of hepatic VOD being 10-60% varies according to diagnostic criteria and conditioning regimens. We retrospectively evaluated the laboratory values and daily progress forms of 65 consecutive cases who underwent hematopoietic stem cell transplantation. Patients and Methods. Sixty-five consecutive patients with various hematologic malignancies underwent HSCT and 68 transplantations were performed (31 autologous SCT, 37 allogeneic SCT with 3 of them being non-myeloablative SCT) between September 2003 to February 2006 with a median age of 42,5 years (range 16-71 years). Three patients received 2nd transplantations, one as a part of tandem allogeneic protocol and two of them as retransplants after relaps of their disease. As a VOD prophylaxis, all patients received ursodeoxyacid, N-acetylcysteine and continous infusion of low dose heparin. The conditioning regimens consisted of cyclophosphamide/TBI, cyclophosphamide/busulfan and cyclophosphamide/busulfan/ fluorara- bine for these patients reported as VOD. VOD was clinically diagnosed with the development of two of the following features: hyperbilirubinemia > 2 mg/dl, hepatomegaly with right upper quadrant pain, and ascite or unexplained weight gain (>5% increase of baseline body weight) within 30 days of transplantation. Patients were said to have multiorgan dysfunction if there was documentation of dysfunction of one other system in addition to liver dysfunction (10,2%) who were diagnosed as VOD were treated with defibrotide intravenously in dosages ranging from 10 to 20 mg/kg per day for a median of 10 days (range 4-25 days). Serious adverse events due to defibrotide was not seen. At diagnosis median bilirubin was 4,6 mg/dl, median weight gain was 8,6%, ascite was present in 45,8% and hepatomegaly a right upper quadrant pain was present in 81,8% of patients. Severe VOD associated with multiorgan dysfunction was present in 2/11 patients (18,2%) with a 100% mortality rate before day 100. Severe VOD was reported to have a mortality rate approaching 100% by day +100 after transplantation which we also experienced in our 2 patients. In general 16/65 patients died (24,6%) and 6/65 (9,2%) of these deaths happened before day +100. Two out of six deaths (33%) happened before day +100 were due to VOD. Complete resolution of VOD was seen in 81,8% with a survival rate of 54,5% at day +100. Conclusion: Although there is still no satisfactory recommendation for the treatment of severe VOD, defibrotide seems to be the best therapy reported with acceptable side effects. In generally complete resolution of VOD was reported as 36-42% in the literature. The favorable complete response rate which we achieved in our series may be due to the early intervention of defibrotide therapy with the diagnosis of moderate to severe VOD in addition to the prophylaxis with ursodeoxyacidic acid, N-acetylcysteine and continous infusion of low dose heparin.

1302

CIRCULATING CFU-GM DURING HEMATOPOIETIC RECOVERY AFTER PERIPHERAL BLOOD TRANSPLANTATION: RELATIONSHIP TO GRANULOCYTE ENGRAFTMENT.

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Hematopoietic progenitor cells (HPC) are circulating in the peripheral blood (PB) before engraftment following auto or allo-PBSCT. It is known that HPC-GM constitutes a part of progenitor cell bodies. In the present study we investigated the kinetics of CFU-GM circulating in PB during the early stages following PBSCT. Patients: Forty seven auto and nine allo-PBSCT recipients were consecutively selected from January 2000 to August 2002 for this study. The median age was 46,5 years (17-69). 20 patients had non-Hodgkin’s lymphoma, 16 myeloma, 8 Hodgkin lymphoma, 7 acute leukemia, 4 solid neoplasms and 1 patient chronic
myeloid leukemia. Cell preparation: 10 ml of PB in heparin was obtained on days 1, 4, 9, 11, 14, 16 and 18 after PBSCT. Mononuclear cells were isolated by Ficoll-Hypaque method. Progenitor assay: A standard methylcellulose colony assay (MethoCultTM GF H4531, StemCell Technologies, Vancouver, Canada) was used for analysing the number of CFU-GM on all of the days. Statistical analysis: Routines within SPSS (Statistical Package for the Social Sciences) were used for the estimates shown. CFU-GM decreased to undetectable on day 4 after transplantation. They reappeared from day 9 to day 18 after transplantation, depending on the patient, along with neutrophil recovery. Figure 1 shows the post-transplant CFU-GM kinetics. We report on the detection of CFU-GM in 13 of 56 patients on day 9 and they number ranged from 2 to 10 per 10 ml PB depending on individual patients. On day 11 we detected CFU-GM in 45 patients, the number of them was 5-12. The number of CFU-GM on day 14 was 5-13 and they were detectable in 54 patients. On day 16 and 18 almost every patients showed CFU-GM colonies -55, the number ranged from 6 to 14 on day 16 and 5 to 18 on day 18. The presence of PB CFU-GM correlates with time of granulocyte recovery (p<0.005). The numbers of CFU-GM PB were similar in the auto and allo-PBSCT. Subsets of CFU-GM were detected during the early posttransplant peri-

The numbers of CFU-GM PB were similar in the auto and allo-PBSCT. Subsets of CFU-GM were detected during the early posttransplant period. They have few changes on days 14 to 18. CFU-GM colonies correlated with granulocyte recovery.

Figure 1. Kinetics of CFU-GM in PB after PBSCT.

1303
SIDE EFFECT OF STEM CELL MOBILIZATION WITH GRANULOCYTE COLONY STIMULATORY FACTOR (G-CSF) ON MORPHOLOGY AND FUNCTION OF LIVER IN MICE
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Mobilization of hematopoietic stem and progenitor cells (HSPC) from the bone marrow into circulation can be induced in patients and animal models by a wide variety of molecules including hematopoietic cytokines, such as recombinant human granulocyte colony-stimulating factor (rH-GSF). A cytokine-mobilized HSPC may ‘home’ to peripheral hematopoietic organs as well as to other sites, for example to liver. However, the morpho-physiology of liver during this process and the safety of G-CSF mobilization are poorly appreciated. To address this problem, the morphology and function of liver in healthy mice after short treatment with G-CSF were investigated. Female (CB6x129)F1 mice 16-20 week-old of age were used. The mobilization of HPSC was induced with 200 mg/kg of rH-GSF injected daily for 8 days. The mice were killed 1, 7 and 28 days after last injection of G-CSF. Peripheral blood was collected and white blood cells were counted. Cell morphology was evaluated on May-Grünwald-Gimsa-stained blood smears. The liver function was monitored by serum bilirubin, enzyme alanin- and aspartat aminotransferase (ALT and AST) levels. Formalin-fixed, paraffin-embedded liver sections were stained with hematoxylin-eosin and Picro-Sirius red for histological examination of livers. Beginning at day 1 after last injection of G-CSF till the end of experiment (day 28) the number of peripheral blood leukocytes did not change and was the same as in control mice. Differential analyses performed on blood smears revealed that the number of mature neutrophils increased significantly after treatment of mice with G-CSF, reaching maximal values on the day 7 after injection of cytokine. Liver in mice receiving G-CSF revealed classic liver lobules with normal architecture and with only mild hepatocellular necrosis. However, on day 7 after the last injection of G-CSF numerous erythrocytes were observed in the lumina of the central and lobular veins. Some erythrocytes and hemosiderin-containing macrophages and the foci of granulopoiesis were visible outside the venous area. Histological examination revealed the presence of collagen synthesis since day 7 (all vessels) till day 28 (mainly portal tract vessels) after mobilization. Distribution and density/frailty of the collagen fibers network was more prominent at the end of experiment. A strong correlation between liver morphology and microsomal enzyme induction was not demonstrated. G-CSF treatment causes morphological, but no functional changes in murine liver. The side effects observed were associated with extramedullary granulopoiesis in liver and with liver blood vessels thrombosis. These adverse effects were partly reversible at 4 weeks post-mobilization. G-CSF stimulates indirectly liver stromal cells to produce collagen, however, time-dependent collagen degradation was not observed in this set of experiment. A long-term follow up is required.

1304
THE EFFECTIVENESS OF PREOPERATIVE ERYTHROPOIETIN IN PEDIATRIC SURGERY
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Backgrounds. The idea about bloodless surgery because of patients' personal or religious concerns, especially in the pediatric population, is challenging. Concerns about the transmission of the human immunodeficiency virus (HIV) have driven the evolution of surgical transfusion practices including the use of preoperative erythropoietin (EPO). Despite the fact that the consequences of transfusion-related diseases are very important issue for pediatric population only a few studies have examined the potential benefits of preoperative erythropoietin in children. Aims. The aim of this study is to assess the clinical efficiency of EPO as preoperative treatment in two children scheduled for heart and abdominal surgery without blood transfusion. Methods and Results. Two children are presented in this study: 15 and 3 year-old boys Jehovah's Witness. They underwent a major surgical procedure without blood transfusion (surgery of the choledochal cyst and corrective treatment of Fallot's tetralogy). Patient that is 15 years old suffered an abdominal pain and presented right upper abdominal mass in association with jaundice eight months before. He underwent clinical, laboratories examinations and ultrasonography. The diagnosis of the choledochal cyst was delivered. Surgery was made at the Department of Pediatric surgery, Medical Faculty in Skopje. The second patient, 3 years old, had discrete microcystosis and fatigue. Diagnosis of Fallot's tetralogy was established by clinical, laboratory examinations, ECG and Echoardiography. A successful surgery procedure was performed at the Pediatric cardiology, Deutsches Herzzentrum- Berlin. Both operations were carried out without the use of blood products through the application of multidisciplinary effort. Preoperative EPO treatment was administrated subcutaneously with 300 U/kg weekly for four weeks (three weeks before surgery and one after surgery). In addition, patients received an oral iron, folic acid and vitamin C supplementation. The effectiveness of preoperative EPO treatment was followed through the RBC count, hemoglobin concentration, hematocrit, platelets and reticulocyte count. Epothelial stimulation was seen with an increase of the reticulocyte count (42 and 61‰) by day 3 of the treatment. The increase of the hematocrit (first patient: from 39.2 to 44%; second patient: from 40.1 to 43.6%), hemoglobin concentration (first patient: from 13.7 to 15.1: second patient: from 12.3 to 14.7g/dl) and increase of RBC count were registered after 3 weeks of treatment with EPO. Both patients didn't receive blood transfusion. EPO caused no adverse reactions. Conclusions. Preoperative EPO treatment has been shown to be an effective alternative to red-cell transfusion in children undergoing surgery. A significant hematopoietic response with no side
Effects was achieved in this study. With respect of this study, but also in accordance with data available from literature, preparative EPO may be used more often in pediatric surgery.

**1305 EVALUATION OF IL-1β, IL-2, IL-4 AND TNF-α IN PATIENTS WITH THE MALIGNANT HEMATOLOGIC PATHOLOGY**

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Interleukins (IL) are the main homeostasis regulating agents and they have very wide spectrum of different biologic effects. They take part in regulation of all components of immune system and execute local immune answer in malignant process but the question about how interleukins realizing their enormous possibilities for anti-tumoral resistance activation and why these possibilities may be diminished in organism with growing tumor mainly is unclear now. Optimization of prognostic criteria and efficiency of treatment for patients with different malignant hematological pathology on the basis of IL-1β, IL-2, IL-4 and TNF-α monitoring. 39 patients (3-61 years) with different malignant pathology (acute leukemia -22 recipients of autoPBSCT (AL)-8 and children ALL group-14); HD-5; MM - 9 were investigated. 25 pts received autoPBSCT. For patients undergoing autoPBSCT the investigation carried out at the following points: before conditioning treatment, after autoPBSCT in time of restoration of hemopoiesis, through ±17 months after transplantation. For children with ALL underwent only for conventional chemotherapy investigation executed in the acute period and in the remission. The evaluation of spontaneous IL-1β, IL-2, IL-4, TNF-α production in supernatants of daily cultures of peripheral blood mononuclear cells were measured by ELISA (Diaclone, France). The control group consisted of 22 healthy persons. Very low IL-1β levels were revealed in all patients groups before autoPBSCT, its level fluctuated within 13-65 pg/ml limits. In the early post-transplant period IL-1β level raised up to 162,28±38,95 pg/ml only in patients with HD (p<0,05), meanwhile in AL group IL-1β level continue to come down (range 7,97-13,47 pg/ml) and in MM group was stable low. IL-2, IL-4 levels in PBSCT recipients group did not differ reliable from those in control in all points of observation except HD. There were higher IL-2, IL-4 levels in these patients before transplantation (79,11±48,5 pg/ml and 3,29±1,21 vs 13,62±0,72 pg/ml and 1,25±0,52 pg/ml in reference group respectively, p<0,05), which became almost normal after PBSCT (20,07±5,77 pg/ml and 1,40±0,58 pg/ml, p<0,05). TNF-α level were low in HD before transplantation and in ALL relapse groups (61,05±17,74 pg/ml and 85,4±25,50 pg/ml vs reference 414,94±101,01 pg/ml). In HD early post-transplant period and in ALL remission group TNF-α level raised up to normal value (401,27±111,34 pg/ml and 320,28±123,04 pg/ml, respectively, p<0,05). The obtained data indicate the preliminary activation of patient’s immuno-competent cells in vivo. The certain differences in ALL, IL-2, IL-4, TNF-α levels in view of disease, its course, treatment with PBSCT and outcome were revealed. The presented data reflects the implications of inflammatory cytokines (TNF-α, IL-1β) in the pathogenesis of AL in children and HD as treatment effectiveness with autoPBSCT implement. We conclude that investigation of these cytokines production may be helpful in optimization of prognostic criteria and treatment effectiveness of the specified pathology.

**1306 ERYTHROPOIETIN SIGNALING INPanCREATIC TUMOR CELL, AR42J: ACTIVATION OF MITOGEN ACTIVATED PROTEIN KINASES AND THEIR EFFECT UPON CELL PROLIFERATION**

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Erythropoietin (EPO) regulates the proliferation and differentiation of erythroid progenitors via its receptor, EPO-R and through various Mitogen activated protein kinase (MAPK) pathways. We reported last year at 10th EHA Congress that high dose EPO could enhance the proliferation of rat pancreatic tumor cell line, AR42J. *Aims.* To extended this study to examine the activation of two MAPKs, namely, extracellular regulated kinase 1/2 (ERK1/2) and c-jun NH2-terminal kinase 1/2 (JNK1/2) in vitro and in vivo. *Methods.* Cells were treated with 5mIU/mL of EPO, 5mIU/mL EPO plus tyrosine kinase inhibitor, 5mIU/mL EPO plus 5mIU/mL of AR42J cells were cultured, exposed to 5mIU/mL of EPO and cell extracts were prepared. These were separated by electrophoresis and subjected to Western blot analysis. EPO induced proliferation was evaluated by 5-Bromo-2-deoxyuridine (BrdU) incorporation method. We found a rap-

**Table 1. Percentage of CD2+, CD4+ and CD7+ granulocytes.**

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<tr>
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<th>CD7+</th>
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<tr>
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**REFERENCES**

**ACKNOWLEDGMENTS**
Backgrounds. G-CSF (granulocyte colony stimulating factor) regulates the production of neutrophils within the bone marrow and also impacts on their function. It is frequently given to patients with leucopaenia or neutropenia caused by various underlying diseases. The treatment with G-CSF is apparently safe although flares in patients with autoimmune diseases and cutaneous vasculitis have been described in the literature. Selected patients with myelodysplasia (MDS) with suppurative infections may be a candidate for G-CSF although there is no data to support their routine use in MDS. Other common side effects of G-CSF are musculoskeletal pain, transient hypotension, deranged LFTs, and transient decrease in blood glucose. Splenic enlargement, hepatomegaly, headache, diarrhoea, epistaxis, alopecia, osteoporosis, and reactions at injection site have been reported.

Figure 1. Rash following G-CSF/histology of rash.

Case history: We report a 55 yr old man who presented to the A/E with a 2 week history of feeling generally unwell with decreased appetite, muscular aches and pains (all joints) and nausea. He had background history of Type II Diabetes Mellitus and hypercholesterolaemia. Blood tests on admission revealed that he had neutropaenia (N=0.6) mild anaemia (Hb = 12) mild thrombocytopaenia and hyponatraemia (Na 129). His bone marrow raised the possibility of early myelodysplasia although his karyotype was normal. An ultrasound scan of the abdomen revealed mild splenomegaly of 14.4 cm. A CT scan revealed a pulmonary nodule 1x2 cm. A lung neoplasia as aetiology of his hyponatremia was queried. However a PET scan was negative. He went to ASCT as ‘salvage’ therapy. No significant differences were achieved in 17 patients with the median follow-up of 14 months (range, 6-27). Eleven patients were died due to relapses of malignancies during the 12 months after ASCT. There were patients with AL of high risk group (n=5), MM (n=2) and resistant lymphomas (n=6) who were under- went to ASCT as ‘salvage’ therapy. No significant differences were found in hematological recovery and transplant-related complications in these patients. Median time to an absolute neutrophil count greater than 0.5x109/l and platelet count greater than 50x109/l was 15 and 20 days, respectively. Lea samples contained 1,92±0.54% CD34-positive cells. The majority of CD34-positive cells were in the G0/G1 phases of cell cycle (67.28%). The mean number apoptotic CD34-positive cells was 2.97%. We noted significantly greater proportion of CD34-positive cells in SM phases of cell cycle in the relapsed patients in comparison with patients who are still in CR (36.1±4.71% vs 22.1±1.53%). Conclusion: Further study would be required in order to clarify the relations of CD34-positive cell cycle state and risk of relapse in patients after ASCT.

Recently, as the constant improvement of chemotherapeutic regime, the development of bone marrow transplantation and biological target therapy, the treatment of leukemia had achieved great progress. However, a major problem in the treatment of leukemia is the development of resistance to chemotherapeutic agents. How to overcome and reverse drug resistance of leukemia cells to chemotherapeutic agents are therefore the critical issues to be solved urgently in the clinic. Recently, it has been shown that quercetin, a Chinese herb, could inhibit the growth of leukemia cells, trigger apoptosis, and even reverse the multidrug resistance of leukemia cells in vitro. But, it remains uncertain that how quercetin can restore the abnormal distribution of DNR in resistant cells. In the present study, we intend to investigate aforementioned effects exerted by quercetin on drug resistant cell line HL-60/ADR in vitro. To investigate the effects of multidrug resistance reversed by quercetin in drug resistant HL-60/ADR cell line in vitro. RT-PCR was employed to detect MRPs expression and elucidate the impact of quercetin on its expression both in HL-60 and HL-60/ADR cells. The subcellular distributions of DNR before and after quercetin insult were measured by confocal microscopy. After being treated with different concentrations of quercetin, there were no apparent regulatory effects exerted by quercetin on MRP1 gene expression in HL-60/ADR cells. However, quercetin could down regulate MRP1 gene expression in HL-60/ADR cells in a dose-dependent manner. In particular, at concentrations of 20 μmol/L
and 40 μmol/L quercetin respectively, there were marked down-regulation of MRP1 gene expression, as compared with mock-treated group (p<0.01). In HL-60 cell line, the DNR fluorescence was mainly distributed in the nucleus, cytoplasm and cell membrane, with nucleus intensely, cytoplasm uniformly and diffusely, membrane continuously staining pattern (Figure A). Compared with mock-treated group, the distributions of DNR fluorescence were not obviously changed after treated with different concentrations of quercetin (Figure B). However, in HL-60/ADR resistant cells, DNR fluorescence was mainly distributed in periphery region of cytoplasm and membrane, the granule was not homogenous, and fluorescence signal was hardly seen in the nucleus (Figure C). Nevertheless, as concentration of quercetin increased, fluorescence signal was gradually increased in the nucleus and cytoplasm. When the concentration of quercetin increased up to 40 μmol/L, the fluorescence intensity almost reached level of that in sensitive cells with diffuse granule distribution (Figure D). Altered subcellular distribution of DNR in resistant cell line was related to MDR gene formation in tumor cells. Quercetin could inhibit MRP1 function and restore the subcellular distribution of DNR in vitro.

**11th Congress of the European Hematology Association**

**1311**

**RETINOIC ACID AFFECTS THE RESPONSE OF V-MYB-TRANSFORMED MONOBLASTS TO OKADAIC ACID**

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**Background.** Okadaic acid (OA) inhibits serine/threonine protein phosphatases 1 (PP1) and 2A (PP2A), thus inducing differentiation and/or apoptosis of various leukemic cell lines in dose-dependent manner. This suggests that PP1 and PP2A phosphatases actively participate in regulation of these processes. Moreover, retinoic acid (RA) affects expression and activity of the PP2A. **Aims.** The aim of this study was to explore the functional interactions of RA- and OA-driven pathways in v-myb-transformed monoblasts BM2. We have previously described that BM2 monoblasts ectopically expressing Jun, RA-receptor (RAR) or retinoid X receptor (RXR) proteins differentiate to macrophage-like cells upon treatment with RA while wild-type BM2 cells do not respond to RA. Results. In this study we found that 10 nM OA induces adherence, cell cycle arrest, phagocytic activity, production of reactive oxygen species and expression of vimentin in BM2 cells. These features that mark differentiation along monocyte/macrophage pathway are enhanced in BM2 cells upon simultaneous treatment with OA and RA. Interestingly, the 20nM OA induced apoptosis is significantly higher than that of BM2 cells as documented by analysis of cell morphology, chromatin condensation, inter-nucleosomal DNA fragmentation and fosfotidylserine translocation. This proapoptotic effect of OA in BM2 cells was inhibited by RA. **Conclusions.** These results indicate that pro-differentiation and pro-apoptotic effects of OA on BM2 monoblasts are differently regulated by RA.

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**1312**

**STUDIES OF PNAS-2, AN ANTI-APOPTOSIS GENE**

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We use gene chip and find PNAS-2 (Gene ID:AF229832) is one of the down-regulated genes upon treatment of As454 in APL cell line-NB4, same result has been reported by other group except the configuration of arsenic sulfide is As2S3 instead of As4S4. Moreover, PNAS-2 expression unchanged in U937 and K562 leukemia cell lines. The hypothetical protein of PNAS-2 shows high sequence similarity to a protein that is thought to be involved in apoptosis, however, there are no studies characterizing this gene. To obtain 5' unknown sequence of PNAS-2, PNAS-2-GFP-fusion proteins expression plasmid was constructed, after transfected to U937 cell line, Western blot analysis was applied to detect GFP fusion proteins; Northern Blot was used to detected the expression of PNAS-2 gene in multi-tissue; Real-Time PCR was applied to detected PNAS-2 expression in patients.

**Background.** We used two splice patterns of PNAS-2 in NB4 cell lines, as F1 PNAS-2 and F2 PNAS-2; both were more than 98% homology to CHMP5, CGI-34 and HSPC177, these genes had a same open reading frame (Figure 1). After transfected GFP fusion protein expression plasmid to U937 cells, we applied Western blot analysis. The results confirmed PNAS-2 could be translated into protein and it was not a pseudogene (Figure 2). Northern Blot was applied in the multi-tissue including heart, brain, placenta, lung, liver, skeletal muscle, kidney, pancreas, spleen, lymph node, thymus, leukocyte, bone marrow and fetal liver, we found no expression of PNAS-2 gene in majority tissues except in placenta (Figure 3, 4). After Real-Time PCR, we found PNAS-2 expression increased in case of acute lymphatic leukemia (ALL) include 71 de novo and 6 relapse when compared with 8 complete remission (CR) patients (p=0.0001) or 57 non-tumorous disease patients (p=0.0003). (Figure 5). There was no statistic difference between each subtype of AL.

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**PNAS-2, A NOVEL GENE PROBABLY PARTICIPATE IN LEUKEMOGENESIS**

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We use gene chip and find PNAS-2 (Gene ID:AE229832) is one of the down-regulated genes upon treatment of As454 in APL cell line-NB4, same result has been reported by other group except the configuration of arsenic sulfide is As2S3 instead of As4S4. Moreover, PNAS-2 expression unchanged in U937 and K562 leukemia cell lines. The hypothetical protein of PNAS-2 shows high sequence similarity to a protein that is thought to be involved in apoptosis, however, there are no studies characterizing this gene. To obtain 5' unknown sequence of PNAS-2, PNAS-2-GFP-fusion proteins expression plasmid was constructed, after transfected to U937 cell line, Western blot analysis was applied to detect GFP fusion proteins; Northern Blot was used to detected the expression of PNAS-2 gene in multi-tissue; Real-Time PCR was applied to detected PNAS-2 expression in patients.

**Background.** We found two splice patterns of PNAS-2 in NB4 cell lines, as F1 PNAS-2 and F2 PNAS-2; both were more than 98% homology to CHMP5, CGI-34 and HSPC177, these genes had a same open reading frame (Figure 1). After transfected GFP fusion protein expression plasmid to U937 cells, we applied Western blot analysis. The results confirmed PNAS-2 could be translated into protein and it was not a pseudogene (Figure 2). Northern Blot was applied in the multi-tissue including heart, brain, placenta, lung, liver, skeletal muscle, kidney, pancreas, spleen, lymph node, thymus, leukocyte, bone marrow and fetal liver, we found no expression of PNAS-2 gene in majority tissues except in placenta (Figure 3, 4). After Real-Time PCR, we found PNAS-2 expression increased in case of acute lymphatic leukemia (ALL) include 71 de novo and 6 relapse when compared with 8 complete remission (CR) patients (p=0.0001) or 57 non-tumorous disease patients (p=0.0003). (Figure 5). There was no statistic difference between each subtype of AL.
We found PNAS-2 highly expressed in 71 de novo AL patients compared with 8 CR patients (p = 0.0001); PNAS-2 also highly expressed in 6 relapse AL patients compared with CR patients (p = 0.0166), but there was no statistical difference between de novo AL patients and relapse AL patients (p = 0.0759). Figure 6. We also found PNAS-2 expression noticeably decreased in 6 AL patients when achieved CR self-compared with onset stage and increased again at relapse, it seems PNAS-2 gene may contribute to leukemogenesis.

1314
REVIST OF DEL(20Q) IN MYELODYSPLASTIC SYNDROMES (MDS): RISK FACTOR ANALYSIS IN MDS PATIENTS WITH DEL(20Q)
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The deletion of the long arm of chromosome 20, hereafter del(20q), is a common cytogenetic abnormality in various myeloid disorders and is known to be a favorable prognostic factor in myelodysplastic syndromes (MDS). However, del(20q) is sometimes found to be associated with disease progression and is detectable as one of additional cytogenetic changes. AIM: In order to ascertain the risk factors in MDS, we analyze 53 patients with MDS showing del(20q). We categorized del(20q) into two types; one is the sole and major del(20q) clone (>50% marrow metaphases) corresponding to genomic instability, while the other is a late appearance of minor del(20q) clone (<50% metaphases) with additional cytogenetic changes representing genomic instability. Of the MDS patients with del(20q) at initial presentation, the negative factors in predicting prognosis on survival are (1) more progressive disease status, (2) any additional cytogenetic changes, or (3) minor del(20q) clone. CONCLUSION: The late appearance of del(20q) at any phase is linked to a significantly unfavorable prognosis, thus indicating the clinical and biological heterogeneity of del(20q) in MDS.

1315
THE 5’KIAA1509/3’PDGFRB FUSION GENE IN MYELOPROLIFERATIVE DISORDERS
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Background. The myeloproliferative disorders (MPDs) are characterized by the abnormal proliferation of one or more myeloid cell types. Unlike the Philadelphia chromosome in chronic myeloid leukemia, there is no specific chromosomal abnormality associated with the MPDs. However, a number of recurrent chromosomal rearrangements, involving a variety of tyrosine kinase genes as PDGFRα, PDGFRβ, FGFR1, and JAK2, have been reported. In this report, we describe a patient with MPD bearing a t(5;14)(q32;q32). As a consequence of this rearrangement, the 5’ region of the KIAA1509 gene was fused to the 3’ portion of PDGFRB. Aims. We performed a molecular cytogenetic analysis by FISH to identify the genes mapping in correspondence to chromosomal breakpoints. Further molecular studies have been carried out to exactly define the breakpoints location within KIAA1509 and PDGFRB genes. The presence of this chromosomal translocation was investigated by the presence of siRNA hybridization (FISH) analysis on additional MPD cases. Methods. FISH experiments were performed with BAC clones specific for KIAA1509 and PDGFRB genes. The 5’ KIAA1509/3’ PDGFRB fusion transcript was detected using a KIAA1509 exon 11 forward primer (KIAA1509-11F) and a PDGFRB exon 11 reverse primer (PDGFRB-11R). The fusion protein domains were identified using the BLAST program (http://www.ncbi.nlm.nih.gov/BLAST). A FISH screening of 12 MPD cases was carried out. Results. FISH cohybridization experiments with RP11-368B7 and RP11-754J8 probes specific for KIAA1509 and PDGFRB genes revealed their involvement in the reciprocal translocation. RT-PCR analysis with KIAA1509-11F and PDGFRB-11R primers produced an amplification product of about 200 bp. The sequence analysis demonstrated that breakpoints were located within KIAA1509 intron 11 and PDGFRB intron 10. According to molecular data, the fusion protein was composed of 2 N-terminal KIAA1509 domains (coiled-coil myosin heavy chain tail and chromosome segregation ATPases region).
and a C-terminal PDGFRB domain (catalytic tyrosine kinase). The use of different primers combinations revealed the absence of the reciprocal 5' PDGFRB/3' KIAA1509 fusion transcript. The FISH screening of 12 MPD patients with BAC clones specific for KIAA1509 and PDGFRB genes did not reveal the presence of other cases bearing the 5' KIAA1509/3' PDGFR fusion gene. The patient with 5' KIAA1509/3' PDGFR fusion transcript, was treated with imatinib and achieved hematological remission; the molecular response is still under evaluation. 

Conclusions. In this study we report the second MPD case with a t(5;14)(q32;q32) bearing a 5' KIAA1509/3' PDGFR fusion gene. One of these pts develop hematological relapse. Maintenance was not changed in 11 pts (10 with probable MR, one - with proved MR) and 4 (36%) of them subsequently relapsed (one with proved MR). Conclusions. According to our data, detecting of PML/RARA in pts during maintenance therapy leads to high incidence of relapse in APL pts. Changing of therapy during MR significantly decreases the probability of hematological relapse [from 36% to 0% (p=0,001)].

1316 
DETECTION AND MONITORING OF CYTOMEGALOVIRUS(CMV) IN BONE MARROW TRANSPLANT (BMT) RECIPIENTS BY REAL-TIME PCR (RQ-PCR)

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CMV has been recognized as the most important viral pathogen in persons undergoing BMT. Monitoring of CMV reaction from latency is critical for these patients. We could detect CMV DNA in this patients by RQ-PCR. For monitoring of CMV reaction. If copy number of CMV was increased, preemptive therapy will be initiated. 51 recipients of BMT (9-51 years) were monitored as weekly intervals until day 100 after transplantation. For amplification of the pp65 gene (UL83) RQ-PCR assay and pp65 Antigenemia method were preformed in parallel with 415 samples. By cloning of this region, we made standards for RQ-PCR. The results obtained by the two techniques were significantly correlated (p<0.01). We could detected 15x101-15x107 copies/2x105 cells by RQ-PCR. 76% of patients developed more than one episode of CMV replication. First positive result of RQ-PCR 13 days earlier than the Antigenemia. After preemptive therapy 16 days (7-21 days) needed to become negative result of RQ-PCR. There was no relationship between death and increase of CMV copy(p<0.419). There is no correlation between copy number of CMV virus and pp65 and WBC count (p<0.624,p<0.422). 

Results. 

RO-PCR was more sensitive than pp65 Antigenemia. After preemptive therapy, negative results of RQ-PCR were the best indicator for determining of successful treatment. Reaction of CMV in our patients mostly endogenous and depend on kind of immunosuppressive therapy. If copy number of CMV increased one log, CMV reaction developed 1.22 fold.

1317 
MONITORING OF MINIMAL RESIDUAL DISEASE AND TREATMENT OF MOLECULAR RELAPSES IN PATIENTS WITH ACUTE PROMYELOCYTIC LEUKEMIA

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Backgrounds. Minimal residual disease monitoring of PML/RARA chimeric transcript is widely used method for detecting of molecular relapses (MR) in pts with APL during hematological remission. However, the necessity of therapy changing when MR is detected is still debated. Aims. We tried to find out whether the PML/RARA detection during hematological remission ultimately leads to relapse of APL and to develop the optimal treatment strategy of MR in APL pts. Materials and Methods. We investigated bone marrow samples by RT-PCR for PML/RARA chimeric transcript in 73 pts with newly diagnosed and morphologically proved APL. Primers synthesis for nested RT-PCR was performed using recommendations of BIOMED-1 Concerted Action (1999). RT-PCR was performed on fresh marrow aspirates of all pts before treatment and periodically (2-Monthly) during all period of therapy (2 years after induction of remission). MR was defined as probable if chimeric transcript was detected once and was not found out by second investigation and as proved when PML/RARA was detected at least twice by consecutive investigations (2-4 weeks). Results. In 69 pts (94,5%) PML/RARA chimeric transcript was revealed during first investigation. 31 (45%) demonstrated bcr1 type of transcript, 38 (55%) - bcr2 type. In 4 pts (5,5%) PML/RARA was not found. During maintenance therapy in 19 of 52 pts (36,5%) MR was detected. In 5 patients from 6 with proved MR and in 3 pts from 13 with probable MR therapy was changed for Ara-C with idarubicine in early MR (12 months from remission induction) or ATRA + Interferon alfa in late onset of MR. No one of these pts developed hematological relapse. Maintenance was not changed in 11 pts (10 with probable MR, one - with proved MR) and 4 (36%) of them subsequently relapsed (one with proved MR). Conclusions. According to our data, detecting of PML/RARA in pts during maintenance therapy leads to high incidence of relapse in APL pts. Changing of therapy during MR significantly decreases the probability of hematological relapse [from 36% to 0% (p=0,001)].

1318 
MONOCONAL ANTIBODY TO CD34 INHIBITS PROLIFERATION AND INDUCES APOPTOSIS OF CD34+ STEM AND MEYLOID CELL LINES

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Backgrounds. Monoclonal antibodies to epitopes of membrane differentiation antigens are widely used for therapeutical targeting of tumor cells. Aims. In some hematological malignancies, however, there is a need for more specific antibodies targeting the surface epitopes expressed on immature hematopoietic cells. Methods. We developed mouse monoclonal antibody of IgG1 class, clone 4H11, reactive with the class III (protein) epitope of human CD34 molecule. We detected the antiproliferative effect of CD34 antibody on human CD34+ stem and myeloid cell lines. Inhibition of proliferation was tested by uptake of triitated thymidine and apoptosis was detected by Annexin-V-Fluorescence kit. Results. Anti-CD34 antibody 4H11 inhibited proliferation and induced apoptosis of CD34+ positive cell lines at the concentration between 1-200 ug/ml after 12, 48 and 72 hours. The anti-CD34 antibody strongly inhibited proliferation and induced apoptosis of all CD34+ cell lines (MOLM-9, JURL, HEL, RPMI 8402) but not control CD34 negative cells. The antiproliferative effect was detected even at the antibody level of 2.5 ug/ml, and the antiproliferative effect was potentiated by simultaneous presence of differentiation inducing cytokines. The expression of CD34 antigen at the surface membrane of tested living cells was not modulated by 4H11 antibody. Conclusions. Based on the results obtained by the ex vivo model system of cultured leukemia cells we suggest that antigenic epitopes expressed on CD34 molecule should be considered as possible new molecular targets for the development of more effective targeted therapy of severe hematological malignancies, especially of immature myeloid lineage. (Supported by grant NR/82333-3 of the Internal grant agency of the Ministry of Health of the Czech Republic).

1319 
ESTIMATION OF THE DIAGNOSTIC VALUE OF MYELOPEROXIDASE INDEX AND LDH IN MEGALOBLASTIC ANEMIA

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Most cases of megaloblastic anemias corresponded to anemia with hyper-segmented neutrophils, macroovalocytosis and very high serum LDH level. Elevated neutrophil myeloperoxidase index (MPXI) may be indicative of a diagnosis of megaloblastic anemia. The aim of this study was to estimate the value of MPXI and LDH in the diagnosis of macrocytic anemia to facilitate the diagnostic algorithm prior to performing any bone marrow aspirate. MPXI and LDH were assessed using the following blood sample obtained prior to any transfusion or medical therapy, and after therapy in 29 patients diagnosed as megaloblastic anemia. MPXI and LDH were assessed using complete blood count (CBC), performed by Technicon H1 (Bayer) instrument. Mean value of MPXI significantly decreased after treatment (20.4, CI95%: 17.25 vs. -0.75, CI95%: -4.27, before and after treatment, respectively). The same significant pattern was also observed for LDH (420, CI95%: 3096-5569 vs. 783, CI95%: 492-1075, before and after treatment, respectively). The proportional diagnostic value (%) was significantly higher when both MPXI and LDH (53 percent, p<0.001) were used together in the diagnosis of Megaloblastic Anemia while the same index was (71 percent, p<0.001) for MPXI and (48 percent, p<0.001) for LDH when they were used alone. MPXI and LDH values may have a diagnostic role on megaloblastic anemia. It might be used as a reliable screening tool before doing any other diagnostic procedure.

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**1320 INTERFERENCE OF HBA1C DETERMINATION BY THE HEMOGLOBIN VARIANT SHELBY**

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Heterozygosity for Hb Shelby was serendipitously discovered in an asymptomatic 48-year-old African American male with a normal complete blood count following ion-exchange high-performance liquid chromatography (HPLC) determination of glycated hemoglobin (Bio-Rad Variant A1c). Aims. Characterization of the Shelby hemoglobin variant and consideration of its interference with HbA1c assessment. The patient had a recent history of blood glucose levels in the normal range (average value = 90 mg/dL), inconsistent with the measured HbA1c level of 12.9% [normal range 4.0-6.0%]. Of note, the interpretive software used for the A1c analysis identified the patient as having sickle cell trait (Hb A/S). Re-assessment of the degree of glycation using a boronate affinity column gave a more clinically appropriate value of 3.9% [normal range 4.0-6.0%]. Additional HPLC analysis using the β-Thal Short Program (Bio-Rad) displayed an unknown peak comprising 26.3% of the total signal with a retention time of 4.84 minutes. Two previously described α-globin variants with similar retention times, O-Indonesia and O-Arab, displayed peaks with distinct conformatonal differences (slender peak bases versus a broad peak base) and associated glycated protein products not observed in our patient. Liquid chromatography-mass spectrometry (LC-MS) on a Finnigan LCQ using electrospray ionization showed a β-globin peak with a molecular weight of 18568 amu and a 758 amu shift from the α-globin peak, isobaric with the normal β-globin chain. However, all three exons of the β-globin gene were sequenced bidirectionally at ARUP Laboratories. Heterozygosity for a nucleic acid mutation CAG→AAG at codon 131 (conferring a GLN→LYS amino acid substitution with zero mass change on MS), consistent with Hb Shelby, was found. This hemoglobin variant is described as unstable. Although asymptomatic, the patient did show a striking clinical symptom. However, some individuals had transient jaundice. In conclusion, this study indicates that diagnosis and classification of G6PD deficiency should be routinely included in the public health care in the Hormozgan province. Moreover, further investigation is required for a better characterization of this disease at the molecular level.

**1321 PREVALENCE OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY IN THE MALE POPULATION OF THE IRANIAN PROVINCE OF HORMOZGAN**

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Backgrounds. G6PD deficiency or Favism is a red cell enzymopathy, which is very frequent in certain areas of the Persian Gulf and in African and Mediterranean countries. Aims. A relatively high incidence of G6PD deficiency and neonatal jaundice carried us to study the prevalence of G6PD deficiency in the Hormozgan province, an area in which about 1.2 million people live. Methods. We randomly selected 816 male individuals aged between 15-20 years living in Bandar Abbas, Haji-Abad, Bandar Lengeh, Rodan, Minab, Jask and Geshm Island. The G6PD activity in the red cells was measured and classified in five categories, from I (the lowest) to V (the highest enzyme activity) and anemia level, according to WHO recommendations. Results. Our survey showed a heterogeneous variety of G6PD phenotypes: 8 cases (0.36%) in class I, 118 cases (14.46%) in class II, 132 cases (16.42%) in class III, 506 cases (62.62%) in class IV, and 1 case (0.1%) in class V. The average hemoglobin levels in class I was 10.2 ±0.6 gm/dL and in other classes was within normal ranges. The geographical distribution of the prevalent rates was as below: class I (1.8%) and class III (18.5%) in the Geshm Island, class II (25.9%) in the Haji-Abad, and class IV (77.1%) in the Bandar Lengeh area. We showed that the mildest clinical symptoms (class IV) were found in the Bandar Lengeh area. The amount of NADP substrate needed to reach half of maximum reaction velocity (KM) was 9.1±7.15 (µmol/L) for class II, 3±0.9 (µmol/L) for class III and 3.6±1.3 µmol/L for class IV while the Km G6P for class II, III, and IV were 31.1±12.8, 44.8±3.4 and 15.6±3.4 µmol/L, respectively. The results of this study confirmed the prevalence of the G6PD deficiency in the Hormozgan province. Moreover, further investigation is required for a better characterization of this disease at the molecular level.

**1322 SEVERE IGA MEDIATED AUTOIMMUNE HAEMOLYTIC ANAEMIA IN HODGKIN’S LYMPHOMA PATIENTS: CASE REPORT**
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Backgrounds. In very rare cases a severe IgA-mediated autoimmune haemolytic anaemia may be observed in Hodgkin lymphoma patients. Here is reported such a case. Case report. A 21-year-old man presented an anaemia with a dyspnea but without fever, loss of weight, pain, sweat, adenopathy, hepatomegaly or splenomegaly - Two years before, a Hodgkin lymphoma with thoracic adenopathy and pericarditis had been diagnosed (Nodular sclerosis, stage II B). A chemotherapy including 6 AVBD cycles and a radiotherapy (50 + 10 GY) had been performed. A remission had followed. At day (D) 0, an anaemia (Haemoglobin 87 g/L, hematocrit 25.8%, and red blood cell (RBC) count 2.4 tera/L) was observed but without leucopenia nor thrombocytopenia. A hyperbilirubinaemia (48 µmol/L) was associated with a sharp drop in the haptoglobin level (lower than 0.2 g/L). There was a high rate of lacticdehydrogenase (LDH) (1007 IU/L), but transaminases, C-reactive protein and fibrinogen levels were normal. A hemolytic anaemia was suspected. Two D later, the anaemia was unchanged and the hemolysis confirmed, but the aetiology was not established. At D8, as the anaemia was getting worse (Hemoglobin 76 g/L), new tests were carried out and a corticotherapy quickly started. The clinical course became satisfactory and no other immunosuppressive therapy or RBC transfusion were needed. Additional anti-IgG and anti-IgM were screened and identified by indirect (IAT) and direct antiglobulin test (DAT). The tests were performed using gel cards. In the DAT, anti-human IgG, IgM, IgA, C3c and C3d Ab were used. For the IgA auto-Ab testing, a second DAT with another anti-human IgA Ab was carried out by gel and tube methods. Results: For the first sample at D2 the IAT was negative and with the routine gel DAT, a negative result was obtained with the anti-IgG and -C3d Ab. Whereas on the second sample at D8, the gel DAT performed was negative with anti-human IgG, IgM, C3c and C3d Ab but strongly positive with anti-IgA Ab. Using the second anti-IgA Ab, a strong positive reaction was also obtained in gel test, but negative in tube. Another gel test was carried out on the D2 sample but with anti-IgG Ab; results were similar to those of the D8 sample. Summary/conclusion: IgA mediated autoimmune haemolytic anaemia is rarely observed in Hodgkin lymphoma patients. When results are negative in the DAT with anti-human IgG, IgM, C3c and C3d Ab and the aetiology not established, a DAT with anti-IgA Ab is then recommended to detect these IgA RBC auto-Ab.

**1323 α-THALASSEMIA CARRIERS IN CRETE: HEMATOLOGICAL AND MOLECULAR STUDY**

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Backgrounds. α-thalassemia is probably the most common monogenic disease. The prevalence of this disorder in different populations varies greatly ranging from less than 1% to over 90%. In the Greek population α-thalassemia appears to be quite heterogenic with frequency of carriers up to 8.3%, a percentage similar to that of α-thalassemia. Aims. To present the hematological and molecular findings of 69 α-thalassemia carriers in Crete, a region of Greece with increased incidence of α-thalassemia. Methods. Erythrocite parameters and erythrocyte membrane and G6PD activity were measured. Fetal haemoglobin (HbF) was measured by High Performance Liquid Chromatography (HPLC), while methyl-violet dye after incubation was used for the detection of HbH inclusions. Serum iron and ferritin concentrations were

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chosen to determine with colorimetric and immunoassay techniques, respectively. GAP, PCR, and hybridization with allelic oligonucleotides (ASO) were used for the molecular analysis for the most common α-thalassemic defects found in the Greek population, the deletional defects – αα, ααα, αααα, and the non-deletional defects IVS1→pentanucleotide deletion, PolyA (AATAAA→AATAAG) and (AATAAA→AATAGA), Hb Agrinio, and Mediterranean. Statistical analysis was carried out with the Student’s t-test. Results. Among the 72 α-thalassemic chromosomes of the 59 α and 10 α-thalassaemia carriers, 44 (61.1%) deletional and 28(38.89%) non-deletional chromosomes were found. The deletions in the deletional chromosomes were the –αα in the 57 chromosomes (84.09%), the ααα in 6 chromosomes (13.64%) and the αα in one chromosome (2.27%). The molecular defects in the non deletional chromosomes were the IVS1→pentanucleotide deletion in 23 chromosomes (62.14%), the PolyA TSaudi mutation in 3 chromosomes (10.72%) and the Hb Icaria mutation in 2 chromosomes (7.14%). All the non deletional defects were related to the βglobin gene. Among the α-thalassaemia carriers, MCV and MCH values were lower in IVS1→pentanucleotide deletion carriers than in –αα deletion carriers (p<0.001 and p<0.001 respectively). No statistically significant differences were noted among the other erythrocytic parameters of these carriers. Summary/Conclusions. A higher percentage of non-deletional chromosomes, a higher percentage of the IVS1→pentanucleotide deletion and a lower percentage of the PolyA TSaudi mutation were observed in the α-thalassaemia carriers in Crete compared to the previously reported percentages found in the general Greek population.

1324

APOLPOLYPEPTIDE E GENOTYPES IN IRANIANS WITH SICKLE CELL DISEASE

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Background. Cardiac abnormality is one of complications in most of patients with sickle cell disease. Apolipoprotein E plays an important role in lipid metabolism. The apoE4 allele has been known to be associated with risk of myocardial infarction and coronary artery disease. Aims. To determine the genotypes of apolipoprotein E and the frequency of apoE4 allele in patients with sickle cell disease. Patients and Methods. Patients studied included 35 sickle cell anemia of which 21 were males and 14 females (age 8-41 years), 15 sickle/β-thalassemia, 8 males and 7 females (age 6-46 years) and 15 sickle cell trait individuals, 9 males and 6 females (age 1-58 years). Sickle cell phenotype was diagnosed in the serum analgesic electrophoresis at alkaline and citrate agar gel electrophoresis at acid pH and by solubility test. Hb A2 was determined by microcolumn chromatography method. DNA was extracted from whole blood using phenol-chloroform procedure. Apo E genotypes were analysed using PCR followed by digestion with Hha I restriction enzyme. Results. Of the six possible apo E genotypes, four were observed in sickle cell anemia patients that were εε/εε (63.5%), εε/εε (17.1%), εε/εε (14.3%) and εε/εε (2.9%). The frequencies of apo E alleles were εε (81.4%), εε (10.0%) and εε (8.6%). In sickle cell trait individuals the order of frequencies of apo E genotypes was: εε/εε (80.0%), εε/εε (6.7%) and εε/εε (13.3%). In sickle cell/thalassemia patients only two apo E genotypes (εε/εε, 96.7% and εε/εε, 13.3%) were exist- ed. Summary/conclusion. It was concluded that the high frequency of apo E4 allele in sickles with sickle cell anemia might increases the morbidity and mortality results from cardiac abnormalities in these patients.

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MOLECULAR CHARACTERIZATION OF GLUCOSE-6-PHOSPHATE DEHYROGENASE DEFICIENCY IN WESTERN IRAN

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Background. Glucose-6-phosphate dehydrogenase (G6PD) deficiency is a worldwide enzymopathy affecting an estimated 400 million people. Its presentation generally occurred as hemolytic episodes after ingestion of fava beans, unripe peas, drug administration or infections. In many populations, the molecular defects responsible for this disease are one or a small group of mutations present at high frequency. Aims. To study the spectrum and frequency of G6PD mutations in school boys of Western Iran. Methods. The studied subjects were 64 G6PD deficient individuals comprised of 52 school boys aging 14-18 years diagnosed during schools screening and 12 children aging 1.5-18 years with history of favism and acute hemolytic anemia. All individuals were Kurds from Kermanshah province. DNA was extracted from whole blood by the phenol-chloroform method. Detection of mutations in coding region of G6PD gene was performed using PCR-RFLP analysis for the characteri- zation of the G6PD Mediterranean and PCR-SSCP technique for the screening of exons 2 through 13. All mutation detected by SSCP were confirmed by an ABI system. Results. The G6PD Mediterranean muta- tion (563 C:T) was detected in 57 males and one female, who was het- erozygous for this mutation giving an allele frequency of 90.62%. G6PD Chatham (1003 G:A) in exon 9 was found in 5 males (7.81%). Nucleotide (nt) sequencing of exon 12 revealed a G:C substitution at nt 1376 (G6PD Cosenza) in one subject (1.56%). All but three individuals with G6PD Mediterranean mutation were male. All the non-G6PD Mediterranean mutation carriers were female. Our findings indicate that the allele frequency of G6PD Mediterranean mutation in Kurds from Western Iran is higher than those from two Fars ethnic groups living in Northern and Southern Iran. Nevertheless they are in strictly accordance with previous report of the prevalence of the G6PD Mediterranean in Kurdish and Middle East population. Also, the strong association of the G6PD Mediterranean mutation and the pres- ence of the polymorphism nt-1311 C:T in the Kermanshah population demonstrate, that the presence of this mutation may be the result of migrations that have taken place through the history.

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ASSESSING ERYTHROPOIETIN IN HEMODIALYSIS PATIENTS: THE IMPACT OF PRO-HEPCIDIN LEVELS

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Background and Aims. Prohepcidin (pro-HPC) is the precursor of hep- cinit (HPC), a liver-derived peptide involved in iron metabolism by blocking its intestinal absorption and its release by the reticuloendothe- lial system. Iron overload and inflammation increase HPC expression, whereas anaemia and hypoxia suppress it. In the present study pro-HPC levels were determined in the serum of hemodialysis (HD) patients and its correlations with iron metabolism markers, C-reactive protein (CRP) and hematocrit (Hct) were assessed. Patients and Methods. 46 HD patients (M/F: 24/22, mean age: 61.1±12.8 years, mean time on HD: 52.2±48.5 months) and 22 healthy volunteers (M/F: 11/11, mean age: 52.2±19.2 years) were studied. Hct, serum pro-HPC, CRP, iron, ferritin, transferrin saturation and soluble transferrin receptors (sTfRs) were measured. Weekly erythropoietin dose and last month intravenous iron dose were recorded. Results. In comparison to healthy volunteers, HD patients had higher serum ferritin (539.77±96.67 vs. 67.58±24.27 ng/ml, p<0.001), sTfRs (0.465±0.173 vs. 0.307±0.109 mg/dl, p<0.001) and CRP (0.904±1.044 vs. 0.186±0.115 mg/dl, p<0.001), lower serum iron (64.45±33.3 vs. 99.77±41.62 ng/dl, p<0.001), Hct (54.1±3.14 vs. 52.8±9.99%), sTFRs (0.307±0.109 mg/dl, p<0.001) and similar transferrin saturation (28.53±13.33 vs. 31.81±11.59, p=ns) and pro-HPC levels (257.46±96.87 ng/ml vs. 234.00±130.82ng/ml, p:ns). In the patients’ group pro-HPC levels were negatively correlated with Hct (p: 0.022) but not with any other of the examined parameters. Multiple linear regression analysis considering age, inflammation, iron adequacy, erythropoietin dose and prohepcidin levels revealed that prohepcidin was the predominant determinant of Hct (p: 0.06). Conclusions. Taking into account the low Hct levels in HD patients of our study, it seems plausible that the pro-HPC levels assessed in this group are inappropriately high. These functionally high pro-HPC levels may belong to the factors that inhibit erythropoiesis in HD patients. On the other hand, the absence of other expected correlations indicates that further studies are needed in order to definitely clarify this aspect.

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ROLE OF ERYTHROPOIETIN IN THE TREATMENT OF PATIENTS WITH SEVERE HEART FAILURE

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Background. Anemia of chronic heart failure (CHF) is multifactor- 

al. Although the biological mechanisms linking anaemia and heart failure are not completely understood, prevalent anaemia is consistent
in patients with severe heart failure and is associated with higher mor-
tality rates. Erythropoietin (EPO) promotes erythrocyte survival and dif-
ferentiation and develops multiple paracrine-autocrine functions that
coordinate local responses to injury. We investigated the effect of EPO
administration in patients suffering from heart failure compatible with
New York Heart Association functional classes III to IV. Twenty-four
anaemic patients, 14 males, median age 62 years (range 47-77) were
studied. 150000 IU EPO (50000 IU per week) were injected i.m. for three
months. The rest of them comprised the control group. Heart failure
functional class was comparable in both groups. Therapy included treat-
meg with digoxin, angiotensin-converting enzyme inhibitors or AII
blockers, carvedilol and diuretics and was not different between the
groups. Hb was welltolerated by all patients. They underwent echocar-
diography in order to evaluate systolic and global left ventricular func-
tion. Ejection fraction (EF) and Tei index (calculated by dividing the sum
of isovolumetric contraction and relaxation time by ejection time) were
estimated at baseline and at the end of the study. Hemoglobin, creati-
nine and electrolytes were measured at baseline and every month later.
Significant increase in hemoglobin values (10.2±0.5 g/dL to 14.2±0.7
g/dL, p<0.01) were observed in the EPO group, but no significant
changes in the control group. Echocardiography showed improvement
in left ventricular systolic and global function in the EPO group (EF
42±5% vs 48±6%, p<0.01, Tei index 0.58±0.14 vs 0.42±0.08, p<0.01),
while echocardiographic indices remained unchanged in the control
group. 2/9 patients of the control group were hospitalized due to de-
compensation of heart failure and none in the EPO group. A slight decrease
in creatinine values in the EPO group was detected at the end of the
study, probably indicating improvement in renal vessels flow, but it was
not statistically significant. EPO significantly improves systolic and glo-
al LV function while creatinine concentration and number of hospitalizations.
Normalization of Hb concentration in patients with CHF may interrupt a vicious cycle, the recently coined cardio-renal-anemia
syndrome. EPO may have a direct positive effect on the heart unrel-
cated to correction of anaemia. Possible mechanisms could be preven-
tion of tissue damage by reducing cell apoptosis and increasing neo-
vascularization.

**1328**

**THE IMPACT OF BONE FORMATION MARKER-OSTEOCALCIN IN PATIENTS WITH β-TALASSEMA**

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The life expectancy of patients with thalassemia has greatly improved
over the last years as a result of regular transfusions and increased com-
plicity with iron chelation therapy, however, this improvement is often
accompanied by a series of serious complications including osteopenia and
osteoporosis. The pathogenesis of these skeletal disorders is multifactori-
al and includes decreased bone mass, increased bone resorption, reduced or absent synthesis of the
β-globin chain of haemoglobin. This disorder is very common in Mediterranean, Middle Eastern, African and
South East Asian populations. The aim of our study was to look for the
mutations in the β-globin gene in the group of unrelated Polish patients with
the β-thalassemia trait. 880 patients (396 men and 484 women)
with microcytosis and no evidence of iron deficiency were examined for
β-thalassemia. The Be Tha Gene 1 Analyte Specific Reagent (ASR) Mod-
ule with the mDx Universal Module was used for detection of the 8
most common Mediterranean β-thalassaemia mutations in a patient’s
DNA sample (Bio-Rad Laboratories). The Be Tha Gene 1 test system is
based on the principle of allele-specific oligonucleotide (ASO) hybridi-
zation. DNA Isolation Kit for Blood/Bone Marrow/Tissue (Roche Diagnos-
tics GmbH, Germany) was used to isolate DNA from leukocytes. Poly-
merase chain reaction (PCR) was used to amplify the fragments of the
β-globin gene. Hemoglobin A2 was increased in 250 patients. In 130
patients A2 was also an elevation of hemoglobin F. 150 patients were
examined for 8 common Mediterranean mutations. 7 different
mutations were detected in 81 heterozygous patients (numbers of patients
with a particular mutation are in square brackets): IVS1-6(T→C) [32];
IVS2-745(C→G) [25]; IVS2-1(G→A) [11]; IVS1-1(G→A) [4]; C677T [2];
G408+A>T [4]; IVS1-1(G→A) [5]. DNA analysis revealed in two patients
(of two unrelated families) with thalassemia intermedia an interna-
tional mutation IVS1-6(T→G) in homozygote stage. Frequencies of individual muta-
tions in Poland were different from those found in Mediterranean and some Central European countries.

**1330**

**CONTRIBUTION OF MTHFR C677T AND A1298C SINGLE NUCLEOTIDE POLYMORPHISMS TO THE GENETIC SUSCEPTIBILITY OF SICKLE CELL DISEASE**

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**Backgrounds.** Methylene tetrahydrofolate reductase (MTHFR) catalyzes
the homocysteine-to-methionine conversion, and a reduction in its activ-
ity leads to elevation in homocystine (Hcy) levels (hyperhomocystein-
eemia), a recognized risk factor for several thrombotic events. The MTH-
FR single nucleotide polymorphism (SNP) C677T results in thermola-
rible enzyme and induces hyperhomocysteinemia, more than the
A1298C SNP. Insofar as sickle cell anemia (SCA) is associated with a
hypercoagulable state, many candidate genes were proposed to induce a
prothrombotic state in SCA patients, including the MTHFR C677T
SNP. Aims. This study addressed the prevalence of C677T and A1298C
MTHFR SNPs among Bahraini SCA patients and control subjects, and
correlate the genotype with changes in Hcy levels. **Method:** this was a
case control study. Study subjects comprised 106 SCA patients (68 male
and 38 female; mean age 15 ± 9.8) and 165 healthy controls (80 male
and 79 female; mean age 27 ± 15.1) all were Bahraini nationals. Mu-
tation analysis was assessed by PCR-RFLP analysis using Hinf I (C677T)
and Mbo II (A1298C). Statistical analysis was performed on SPSS v. 13.0
statistics software. Fisher’s exact test and Pearson’s χ2 test were used to
assess inter-group significance, set at p < 0.05. Results. The frequencies
of the C/C variant of the A1298C but not C677T T/T (p = 0.67) SNP were
significantly higher in patients than controls (p = 0.03; RR = 2.55). Differences between patients and controls
in C677T and A1298C distribution were also noted in haematocyte
distribution. Elevated 66% C677T and 23% C677T carriers in patient
(p = 0.05; OR = 2.589). While they were elevated in 6777T (but not C/C)
carrier, Hcy levels were comparable between patients and controls. Sum-

mary/conclusion: Results from this study showed that A1298C, but not C677T SNP, was associated with SCD. While the mechanism underlying C/C effect was not addressed here, it’s not likely to involve changes in Hcy levels, since Hcy level was comparable between patients and controls.

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VITAMIN B12 DEFICIENCY AND THROMBOSIS
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Backgrounds. Hyperhomocysteinemia is a risk factor for arterial and venous thrombosis. Acquired hyperhomocysteinemia may cause thrombosis in vitamin B12 deficiency. Aims: To evaluate the thromboembolic events in patients with vitamin B12 deficiency. Methods: One hundred forty-three patients (64 female, mean age 59±13 years) with vitamin B12 deficiency (vitamin B12 level < 200 pg/ml) were enrolled to this study. In control group, there were 129 healthy persons (62 female, mean age 58±8 years). Upper gastrointestinal endoscopy was performed to 102 patients. Antibody to parietal cells and the levels of homocysteine were examined in 78 and 36 patients, respectively. In last three years, arterial and venous thromboembolic events were detected. χ² and student-t test were used in the comparison of two groups. Results. Thromboembolic events were detected in 9.8% of the patients with vitamin B12 deficiency. The sites of thromboembolic events were coronary arteries in 5 patients, deep venous and cerebrovascular thrombosis in two patients, respectively. There were thromboembolic events in 3.9% of controls. These rates were not different in two groups (p>0.05). The levels of homocysteine were high (> 20 mMol/L) in all of 36 patients. Conclusion. Thrombosis was not higher in vitamin B12 deficiency. Although we did not examine the levels of homocysteine in all of the patients, hyperhomocysteinemia may not contribute to thrombosis. More extended studies should be done in this topic.

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VALIDATION OF A DEDICATED HPLC METHODOLOGY FOR THE IMPLEMENTATION OF NEONATAL SCREENING OF HEMOGLOBINOPATHIES. A TOOL FOR PRIMARY AND SECONDARY PREVENTION IN A MULTI ETHNIC SOCIETY
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Backgrounds. Hemoglobinopathies (HbPs) are the most prevalent recessive disorder in Man. At least 300,000 affected children are born each year from parents who are both healthy carriers, most of them in endemic countries. Migrations have drastically changed the composition of the population in non-endemic countries where this disease used to be growing. The number of patients in The Netherlands will double in the next decade if no prevention is offered. Although only partially effective for primary prevention the Ministry of Health recently decided to include the screening for hemoglobinopathies, and of sickle cell disease in particular, in the existing neonatal screening program. Aims: We have validated the Variant Newborn Blood Screening (Vnbs) HPLC apparatus (Bio-Rad) to determine if and how it could be used at a maximum of diagnostic efficiency in a national screening program for HbP. We intended to study how neonatal screening on HPLC could allow the implementation of secondary (morbidity) prevention to be planned in advance of the second semester of life, when the diseases will start manifesting. We also intended to study how primary prospective and/or primary prospective prevention could be efficiently offered whenever an affected or a carrier neonate is detected and the parents are informed and referred to a genetic center for counseling and eventually prenatal diagnosis. Methods. We have created fresh artificial standard blood samples and we have used natural cord blood samples (CBS) to test the diagnostic confidence and the sample conditions before and after spotting aliquots on paper to be tested at increasing intervals of time up to a maximum of 3 weeks. Samples eluted from dry 3-mm paper discs were analyzed on HPLC, according to the manufacturer’s instructions and in several modified manners. Results: were compared with the expected patterns for their diagnostic quality and stability. Results. All current abnormal Hb’s involved in SCD were identified using the artificial standard blood samples. In addition 94 natural CBS were analyzed on which we were able to identify Hb Bart’s, and Hb S traits. DNA analysis confirmed the association of Hb Bart’s to a -α deletion. The samples spotted on paper degenerated rapidly. However, the interpretation of the results was still reliable on 15 days old dry samples, which period falls well within the boundaries of the screening program. The integration system of the Vnbs is not measuring the HbA% with the precision necessary to make an educated prediction upon a possible thalassemia carrier. We are testing at this moment possible alternatives. Summary/Conclusions: The (Vnbs) HPLC apparatus recognizes with sufficient confidence all common Hb variants in heterozygous and homozygous state including Hb S/S (SCD) and B-thalassemia major. This will enable pre-symptomatic genotype/phenotype determination and treatment planning for both diseases with a considerable gain in morbidity prevention and state of the art treatment. Moreover, obligatory or potential couple at risk can be immediately referred to a genetic centre for analysis, counseling and eventually primary prevention in a following pregnancy.

1333
A SIMPLE, ACCURATE METHOD FOR THE ESTIMATION OF THE ERYTHROCYTE SEDIMENTATION RATE
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Backgrounds. Measurement of the erythrocyte sedimentation rate (ESR) is a helpful indicator of the presence and extent of inflammation and its response to treatment. ESR is influenced by proteins of acute phase response and anemia which may be present in such situations. Aims: To validate a new easy, accurate, fast and low cost method for the estimation of the ESR. Methods. We studied 65 venous blood samples for ESR, 35 were taken from women and 30 from men. The method we used is that of Westergren as recommended by the International Council for Standardization in Haematology, and the anticoagulant we used was trisodium citrate in the proportion of 1 to 4. Regression analysis was used for describing the behavior of the ESR of the patients in an hour time period. Measurements of their ESR were recorded every ten minutes. Results. by using the control measurement of the ESR at 20 minutes, the blood samples were classified into three homogeneous groups (group-1: 0-5 mmHg at 20 min, group-2: 6-10 mmHg at 20 min, group-3 more than 10 mmHg at 20 min) and a family of regression curves was fitted to the empirical data describing the relationship of the ESR on time. The linear model was chosen as the simplest with good fitting precision in the study. Conclusion: the constructed linear curves within the established groups of the patients enable the estimation of the ESR values at 60 minutes period, with only one measurement at 20 minutes.
Backgrounds. Hemoglobinopathies, which include β thalassaemia, are a common group of genetic disorders prevalent in tropical and Mediterranean regions. They are co-incident with malaria suggesting that these disorders provide a selective advantage in malaria endemic areas. β thalassaemia is characterised by deficient or absent synthesis of the β globin protein arising due to either point mutations, of which more than 200 have been described, or deletions of the β globin gene. Many of the common point mutations are associated with a particular haplotype and haplotype analysis indicates they have common origins. Intriguingly, a low frequency of β thalassaemia mutations have been described in non-tropical populations such as Britain and although most of these mutations are of non-native origin, some are novel. Previously several Irish cases of β thalassaemia have been documented and a number of individual mutations have been identified in the Northern Ireland population but the molecular basis in all cases has not been investigated. Aims. To discover if the β thalassaemia trait in County Down was associated with common or unique mutations and to perform haplotype analysis to indicate the origin of the common mutations detected. Methods. DNA samples from 23 individuals were screened for base changes in the β globin gene using PCR-direct sequencing. Haplotype analysis was performed using seven polymorphic sites of the β-globin gene cluster on chromosome 11. Markers were amplified by PCR and products were analysed by restriction digest. Haplotypes were constructed according to Örkwén et al. [Nature 396 (1982) 627]. Results. Sequencing the β-globin gene revealed that fourteen individuals possessed two common Mediterranean mutations, a C to T change at codon 39 in exon 2 and a G to A change at base 110 in intron 1. Both mutations were present on Haploype I, indicating a non-native origin. A further group of seven individuals shared a G to A change at base 850 in intron 2. This mutation has been previously described in a Canadian family of Scottish-English descent (Curtuk et al. Hematology 1995, 49:207). Finally, two novel β-thalassaemia mutations were detected. The A to C change at the initiation codon would cause defective mRNA translation, while the deletion of G from the last base of codon 109 would result in frameshift mutation. Summary. The two Mediterranean mutations, having arisen on Haploype I, are of non-native origin and may have been introduced into the Northern Ireland population as a consequence of European trade. It is probable that the IVS2-850 (G to A) detected in this study shares a common origin with the family described by Curtuk et al. Finally, it remains to be confirmed if the novel mutations have arisen de novo in Northern Ireland.

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COMPARISON BETWEEN TECHNICON H3 AND COULTER GENSTECNOLOGY IN SPHEROCYTES DETECTION
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Spherocytes are not found in peripheral blood unless conditions such as hereditary spherocytosis, autoimmune haemolytic anaemia, microangiopathy, haemoglobin C or Clostridium sepsis are present. The value of the quantification of MCHC by laser techniques ox% Hyper (% of red cells with Hb concentration >410 g/l) as measured by Technicon cell counters has already been shown. When reticulocytes are measured by the Coulter Genstec counter, the sample is subjected to a low osmolarity and a parameter known as MCVE is generated. Under normal conditions MCV is higher than MCVE but the reverse is seen when spherocytes are present. To determine if the measurement of MCVE is useful to detect spherocytes when compared to ox% Hyper. To evaluate calculated CHCM by both counters and compare it with CHCM measured by laser (Technicon H3). We have simultaneously analysed 44 samples with a % Hyper >4 by both counters (Technicon H3 and Coulter Genstec). Additional studies showed the following abnormalities in 23 of the samples: 11 were hereditary spherocytosis, 8 autoimmune haemolytic anaemias, 2 haemoglobin C and one case of microangiopathy. Statistical analysis was performed with SPSS software, using Pearson correlation and receiver operating characteristic (ROC) curves. Parameters measured by both counters are shown in table attachment. The CHCM H3 values are bigger than calculated CHCM Gens. ‘MCV-MVCE’ median was positive. We have correlated ox% Hyper to the rest of parameters; the correlation was highly significant (p<0.002) with all the parameters but CHCM Gens. When we focused on the specificity of the technique, the correlation using ROC curves, the values of area under the curve (AUC) were 0.808 for ox% Hyper and 0.766 for MCV-MVCE. There was significant correlation between the values of MCV-MVCE, measured by the Coulter Genstec, and the presence of spherocytes. However, both sensibility and specificity of this technique were lower than that shown by ox% Hyper measured by the Technicon counter.
1337
STUDY OF ERYTHROCYTE MEMBRANE PROTEINS BY SDS-PAGE 10 YEARS EXPERIENCE
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Backgrounds. In the majority of Hereditary Spherocytosis (HS) and Hereditary Elliptocytosis (HE) cases the diagnosis can be made on the basis of clinical/ family history, red cell indices and osmotic fragility screening test. Quantification of erythrocytes membrane protein electrophoresis by SDS-PAGE can be useful in complex cases.

Objectives. To identify the most common protein defects in our population and to analyze the usefulness of erythrocytes membrane protein electrophoresis in the diagnosis of patients with anemia and/or hemolysis of unknown or multiple etiologies, we made the retrospective analysis of 584 non familial cases. Materials and Methods. 584 EDTA blood samples from Centro Hospitalar de Coimbra and from other Hospitals in Portugal and Spain. SDS-PAGE was performed according to J. Delaunay protocols. The reasons for the study were divided in 12 groups as listed on the Table. Results. 63% of HS cases have combined Ankyrin/Spectrin/Pr 4.2 deficiencies, 34% combined Band 3/Pr 4.2 deficiencies and in 4% a single Pr 4.2 reduction was detected. Pr 4.1 reduction was found in 56% of the HE cases and the remainder 44% had Spectrin αβ reduction. In 14 cases with HA of multiple etiology we detected Spectrin αβ reduction in four and Pr 4.1 reduction in seven. Among the 48 samples with HA of unknown etiology, one was HS and four were HE. In 18 samples referred as possible CDAs, two had Band 3 abnormal mobility. In the EMPD, SAO and AHAI the electrophoretic profile was similar to the normal controls. No abnormalities were observed in the group of samples referring investigation of anemia Conclusion: In HS and HE the relative percentage of protein deficits involved are similar to the described for other European populations (Delaunay et al., 1995, Eber et al., 1996). In our experience, SDS-PAGE electrophoresis can be useful for the diagnosis of CDA type II, when an EMPD is suspected, in HA of complex etiology and when the results of screening tests were equivocal or borderline or the clinical phenotype is heterogeneous among the affected family members. If no spherocytes or eliptocytes are observed in peripheral blood smears, erythrocytes membrane protein electrophoresis carries no benefit.

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SPECTRUM OF ANTINUCLEAR ANTIBODIES IN SICKLE CELL DISEASE PATIENTS FROM OMAN
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Backgrounds. Sickle cell disease (SCD) is a significant public health problem in the Sultanate of Oman. Although rare, it is not infrequent to find the SCD associated with systemic lupus erythematosus (SLE) or other connective tissue disorders. Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease characterized by a variable clinical picture, a large range of clinical and serological manifestations and a relapsing-remitting course. The disease is very variable in severity. The complexity of the clinical picture of SCD can be increased by the simultaneous presence of manifestations attributable to disease activity, chronic damage, co-morbidity, especially infection or the co-existence with SLE.

Figure 1. Clinical and Immunological characteristics.

Aims. To evaluate the prevalence of antinuclear antibody [ANA] positivity in sickle cell anemia patients. Methods. The study enrolled 67 SCD patients attending the Hematology services at the Sultan Qaboos University Hospital. All patients were explained the objectives of the study and gave an informed consent. Anticoagulated blood was collected for a full blood count and hemoglobin electrophoresis by high performance liquid chromatography. Blood was also collected for a battery of autoantibody profiles including anti-nuclear antibodies, double stranded anti-DNA antibodies and anticardiolipin antibodies, etc. All these tests were also performed in 107 healthy blood bank donors after informed consent, and in 35 sickle cell trait subjects as controls. Results. A total of 67 patients [31 males;36 females] with the mean ± SD age of 24.6±4.9yrs[Range 11-47] formed the study group. ANA was documented to be positive in 16/67 cases [24%] amongst the SCD patients. ANA positivity was noticed in 10/107[9.35%] normal subjects and 6/35[17.2%] sickle cell trait subjects. 6 of the 16[37.5%] SCD patients satisfied the revised classification criteria [minimum 4] for SLE of the American College of Rheumatology.[Figure] Three patients had lupus anticoagulant, five had ACA positivity, one had Bechets, one had anti-thyroid antibodies and one had anti-red cell antibodies. Discussion: The study has demonstrated the prevalence of APA positivity in normal subjects, sickle cell trait subjects and patients with SCD in Oman. The overall APA positivity was observed to be about 24% which is considerably high. The prevalence was also twice as high in females as seen in the males. Furthermore, a significant number of these patients also had multiple autoantibodies.

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LYMPHOID EXTRAMEDULLARY BLAST CRISIS OF CHRONIC MYELOID LEUKAEMIA SIX YEARS AFTER ALLOGENIC TRANSPLANTATION
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Extramedullary disease (EMD), also called granulocytic sarcoma, following allogeneic hematopoietic stem cell transplant (allo-HSCT) in patients diagnosed with chronic myeloid leukaemia (CML) is an infrequent event. According to a retrospective analysis performed by the European Group for Blood and Marrow Transplantation the incidence rate for this complication was 0.22%. Considering that lymphoid transformation accounts for only 20-30% of all blast crisis events in CML, lymphoid EMD remains a relatively rare phenomenon. Reviewing the literature we found only four cases of lymphoid EMD relapse after an
SIDEROBLASTIC CHANGES CAN BE RECOGNIZED IN THE ERYTHROGRAM

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New generation hematology systems supply essential information on blood cells (BC) complementing microscopic cell examination. The scatter plots of red blood cells (RBC), called erythrograms produced by the ADVIA 2120 cell counter gives a visual representation of RBC characteristics. Using a pair of threshold gates on each axis, nine areas are defined according to the cell volume (fl) and the hemoglobin contents (g/dl). Normal RBC are distributed in the central quadrant. The number of cells outside these thresholds gives an accurate percentage of macrocytic, microcytic, hypochromic and hyperchromic RBC. In diseases such as iron deficiency or thalassemia the erythrogram shows a characteristic pattern and is commonly used for diagnostic approach. We observed in patients, mainly with myeloid neoplasia with ringed sideroblasts (RS) in the bone marrow a particular erythrogram pattern with a broad distribution of the RBC, a marked variation in RBC size and hemoglobinization. From the central quadrant an abnormal RBC population shifts on an imaginary axis to the lower-left quadrant representing a shift that advances into the microcytic and hypochromic quadrants (Figure 1). To confirm whether this particular erythrogram was predictive for bone marrows sideroblastic changes, we compared retrospectively the erythrograms of patients with RS to a group of myeloid neoplasia.

In conclusion: Sideroblastic changes can be recognized in the erythrogram. Indeed, despite myeloid neoplasia with RS are a heterogeneous group of diseases they have a common pattern of RBC distribution that can be considered as a kind of fingerprinting for sideroblastic changes with a high predictive value allowing a straightforward diagnostic approach in clinical practice.

The erythrogram was typical in 17/21 patients with RS and in 0/30 patients with No RS (p<0.0001). The positive predictive value for sideroblastic changes was 100% and the negative predictive value was 88%. Despite the RBC indices comparison showed statistical significance in some variables, they were no specific enough to identify sideroblastic changes. In the group with RS, mean cell hemoglobin was lower (median 30.8 versus 33.6 fl), RBC distribution width was higher (19.3% versus 16.5%), the percentage of hypochromic RBC was higher (5.3% versus 0.9%) and hemoglobin content of reticulocyte was lower (53 versus 57 pg) as compared to No RS patients (p<0.05). This last index was useful to rule out iron deficiency in RS group as a cause of hypochromatic RBC changes, since in contrast to iron deficiency it was not decreased. In conclusion: Sideroblastic changes can be recognized in the erythrogram.

EPIGENETIC DATA ON MYELOID-PLASTIC SYNDROME PATIENTS FROM A ROMANIAN SINGLE CENTER

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Background. Since the World Health Organization (WHO) recognized MDS as a disease entity only starting with 1997, epidemiological data on MDS cannot be obtained from official statistics on morbidity and mortality and have to be extracted from specialized registers. We present the first romanian study on the incidence and characteristics of MDS, based on the data existing in Fundeni Clinical Institute, Bucharest, the greatest hematological department in Romania. Method. The MDS files at diagnosis of the patients admitted during the period 1980-2005, recorded in the registration forms provided by the MDS Foundation (USA), represented the primary data-base. The hematological data of the MDS patients included in the registry were re-evaluated and classified according to French-American-British (FAB) criteria. The distribution by sex, age groups, subtypes and the annual number of new cases were analysed comparatively with other reference studies. Results. Four-hundred and twenty four cases of MDS were identified. The distribution between sexes was relatively balanced with a slight global preponderance of males (M/F 1.26), except for refractory anemia with excess of blasts (RAEB) 1.94. The mean age at diagnosis was 62.5 years (16-90). Most of the patients (60.6%) belonged to the group of age 61-90, where all the subtypes of MDS had the highest rates. A noticeable proportion (17%) had ages below 50 years, 25% of which in the range 16-30. On the other hand, few cases (4%) were above 81. Patients with refractory anemia (RA) and refractory anemia with ringed sideroblasts (RARS) accounted for 44.5% of all cases (RA 29%, RARS 15.5%), RAEB and RAEB in transformation 33%, chronic myelomonocytic leukemia 5.6% and unclassified 16.7%. The annual number of new cases was constantly low during the period 1980-1989, but increased dramatically from 11 cases/year in 1990 to a maximum of 48 cases/year in 1999, showing a...
certain decrease afterwards. The subtypes with the most important increase in time were RA and RARS. Conclusions. This study indicates an actual increase of the number of MDS cases in Romania over the investigated period of time. Particularly, a noticeable proportion of young patients and a low proportion of patients ≥81 years have been found, which make our findings closer to the Asian than to the Western MDS epidemiological results.

**1342**

**SINGLE PEG-FILGRASTIM INJECTION AFTER FLURADARINE-CYTARABINE BASED REGIMENS FOR TREATMENT OF POOR PROGNOSIS MYELODYSPLASTIC SYNDROME (MDS) AND ACUTE MYELOID LEUKEMIA (AML): PRELIMINARY DATA ON HAEMATOLOGIC RECOVERY**

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Backgrounds. Response of poor prognosis MDS and AML to conventional chemotherapy (CT) is unsatisfactory. Regimens comprising Fludarabine and Cytarabine (FLA), with or without Idarubicin, have shown promising results in induction of complete remission (CR), with a favourable toxicity profile. In FLA regimens filgrastim is administered from day 0 to day 5 to induce cell cycling and therefore sensitization to CT, then from day 12 to enhance recovery of neutrophils. Peg-filgrastim is a covalently bound conjugate of filgrastim and monomethoxypolyethyleneglycol. It has a longer elimination half-life than the unconjugated filgrastim because of decreased serum clearance. After standard chemotherapy for non-myeloid malignancies, one dose of Peg-filgrastim showed to be equivalent to daily filgrastim in enhancing neutrophil recovery, and the single injection was largely preferred by patients (pts).

From March 2005, in MDS/AML pts we started to administer a single dose of Peg-filgrastim at day 12 of FLA regimens instead of daily unconjugated filgrastim. Aim: to evaluate the efficacy and cost effectiveness of a single Peg-filgrastim injection given at day 12 from the beginning of FLA regimens, in poor prognosis MDS and AML pts. Methods. From March 2005 to December 2005 13 FLA cycles with Peg-filgrastim s.c. injection at day 12 have been administered to 10 pts, at our Institute (Group PEG); neutrophil and platelet absolute count have been monitored daily from day 0. Data on haematological recovery after 53 FLA cycles with unconjugated filgrastim (dosage: 300 mcg/sqm/day) in 36 pts, period January 1999-February 2005, have been retrieved from our database (Group NO-PEG). Filgrastim has been administered until neutrophil count >500/mm3. Group PEG: median age 66 (range 49-73); diagnosis of MDS=3, AML=6, granulocytic sarcoma=1; status pre-FLA: CR1=5, CR>1=2, NOCR=6. Group NO-PEG: median age 56 (range 22-69); diagnosis of MDS=22, AML=14, granulocytic sarcoma=0; status pre-FLA: CR1=16, CR>1=0, NOCR=27. Results. Data on haematologic recovery are shown in the table.

<table>
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<th>Group PEG</th>
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<td>median</td>
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<tr>
<td>neutrophils &lt;500/mm³ (n° of days)</td>
<td>17</td>
<td>7-27</td>
<td>16</td>
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<td>neutrophils &gt;500/mm³ (day from start of CT)</td>
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<td>Platelets &lt;20000/mm³ (n° of days)</td>
<td>18</td>
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<tr>
<td>Platelets &gt;20000/mm³ (day from start of CT)</td>
<td>22</td>
<td>18-23</td>
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n.e.=not evaluable.

A single injection of Peg-filgrastim has been administered in 12 out of 13 cycles in Group PEG; in one case a second injection has been administered at day 32, for delayed recovery. The mean number of vials per cycle of unconjugated filgrastim administered to Group NO-PEG has been 21 (range 6-57). Conclusions. According to our preliminary results, a single Peg-filgrastim injection after FLA regimens results in haematologic recovery comparable to that achieved by daily unconjugated filgrastim; therefore, it would safely spare patients to receive multiple injections. Moreover, regarding the high number of filgrastim vials required to enhance neutrophil recovery in our pts, the conjugated formulation could be favourable also in terms of cost-effectiveness. These preliminary data must be confirmed in a larger population of pts.

**1343**

**ANALYSIS OF CASPASES GENES EXPRESSION IN THE BONE MARROW OF ADULT DE NOVO MYELODYSPLASTIC SYNDROMES (MDS)**

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Backgrounds. Myelodysplastic syndromes cover a range of clonal stem cell disorders characterized by ineffective hematopoiesis which has been associated with excessive intramedullary apoptosis of hematopoietic cells. Caspases constitute a family of cystolic proteases which are the effector molecules of apoptosis. Aim: The aim of the present study was to examine caspases and granzyme B expression and the degree of apoptosis in the bone marrow of adult de novo myelodysplastic syndromes and to correlate our findings with clinical parameters and prognosis.

Methods. We studied 81 cases of MDS including 7 RAEB-t, 9 RAEB, 4 CMML, 7 RA and 4 RARS according to FAB criteria. The degree of apoptosis was determined by flow cytometry using the Annexin method on fresh bone marrow mononuclear cells. mRNA was extracted and the expression of caspases 1, 2, 5, 6, 7, 8 and 9 and Granzyme-B was determined using a multiplexed RT-PCR assay system (Ribobio, BD Biosciences). A pool of RNA from normal bone marrow mononuclear cells was used as a normal control. The expression of each gene was compared to that of two housekeeping genes (GAPDH and LSU) using the Image Master analysis software. The level of each gene expression was compared to that obtained from the normal pool RNA. A ratio was obtained and two groups were generated with values > or <1. The expression of the genes and the degree of apoptosis were analyzed taking into consideration haematological parameters, the FAB classification and the IPSS value. Results. The median value of apoptosis for all MDS cases was 4/7. Apoptosis in the low risk group was higher but not significantly different from the high risk group (FAB RARS, CMML and RAEB). More cases were in the low risk group (FAB RAEB, CMML and RAEB-t). Conclusions. 81 cases of MDS were studied. The median value of apoptosis in the low risk group was higher but not significantly different from the high risk group. Larger number of cases need to be examined to draw definite conclusion about the role of these apoptosis regulatory genes in the pathogenesis and prognosis of MDS.
BM lymphocytes of patients with MDS, as there is a controversy between research groups regarding the lymphoid lineage participation in the pathogenesis, diagnosis and/or in the prognosis of MDS. Following the new classification of MDS by the World Health Organization (WHO) it seems challenging and interesting to investigate, apart from MDS, a group of patients with CMML, which has been classified by WHO as the new group of Myelodysplastic/Myeloproliferative Disorders (MDS/MPD). Methods. BM samples from 32 patients with MDS (n=15), MDS/MPD (n=12), and MDS/AL (n=7) and 5 BM from healthy individuals, as a control group, were analyzed by multiparametric 3-color flow cytometry using an extensive combination panel of monoclonal antibodies (CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD11b, CD11c, CD13, CD14, CD15, CD16, CD19, CD20, CD25, CD34, CD36, CD41, CD56, CD64, CD66b, CD117, HLA-DR, KORSA, MPO/LF and TdT) in the gate of the lymphocytes by the histogram of SSC=1 (expression of CD45). Results. The MDS patients were characterized by the following statistical significant findings in comparison to the control group: a) a decrease of the lymphocytes mean SSC (59,42±5,62 vs 67,00±15,52, p=0,001) and b) a decrease of the fluorescence intensity of CD45 in lymphocytes (43,98±15,99 vs 65,64±17,12, p=0,035). The MDS/MPD patients compared to the control group were characterised by: a) a decrease of the CD38+ (31,01±25,87 vs 42,34±25,06, p=0,013) and b) a decrease of CD56+ (5,80±6,69 vs 20,45±16,72, p=0,056) lymphocytes percentage. The MDS group in comparison to the MDS/MPD group showed a statistical significant decrease of the lymphocytes mean SSC (59,42±5,62 vs 70,83±17,02, p=0,014) along with a decrease of the percentage of co-expression of CD3/CD16/CD56 (5,80±3,86 vs 11,58±6,72, p=0,026). When MDS/MPD group was compared with MDS/AL a decrease of 1 lymphocytes (CD2+; 58,59±3,80 vs 68,06±5,57, p=0,059) and an increase of B lymphocytes (CD19+; 12,72±5,23 vs 7,34±6,08, p=0,014 and CD19+; 12,67±3,55 vs 7,36±5,64, p=0,039) were observed. It should be noted that myeloid markers expression in the lymphoid populations groups didn’t show any differences. Summary/Conclusions. The above-mentioned statistical significant findings indicate the importance of further study of this cell lineage in MDS and MDS/MPD cases to answer several questions such as the decrease of lymphocytes side scatter and CD45 expression indicative of lymphocyte immaturity? As this is an ongoing study, more cases will possibly clarify the disturbances of lymphocytes and their significance in this group of patients.

We have studied the methylation status of the differentially methylated region (DMR) of GTL2 promoter in order to detect epigenetic alterations of DLK1/GTL2 gene. Methods. We have studied 8 patients, 6 males and 2 females, with myelodysplastic syndromes (MDS) classified according to the FAB system; 2 patient with RA (25%), 3 with RAEB (37.5%), 2 with RAEB-T (25%), and 1 with CMML (12.5%). Median age was 68-4 years (range 38-88). Cytogenetic analysis: 2 patients had complex (more than 3 structural abnormalities). 4 patients had apparently normal karyotypes, 1 had 45,XX,-karyotype and 1 had 47,XX,+8 karyotype. None of the patients had ever received therapy with hypomethylating agents. DNA methylation pattern was determined by methylation-specific PCR of samples previously subjected to bisulfite-treatment, according to preestablished procedures. Subjects who have undergone bone marrow aspiration for diagnosis of thrombocytopenia, and after we had excluded hematological malignancies, served as controls. Results. We have studied the methylation pattern in both blood and bone marrow. The normal pattern consists of 2 bands (alleles), namely one corresponding to the methylated paternal allele, (size 160 bp) and one corresponding to the unmethylated maternal allele (size 120bp). We have found that alterations of the DMR were present in 4 (50%) of the patients studied: 2 (25%) had an abnormal methylation pattern in both blood and bone marrow samples and 2 (25%) others presented the same abnormal methylation pattern only in blood samples. No alteration of the methylation pattern was observed in the remaining 6 bone marrow samples. In the remaining 4 samples only the methylated allele was present. Summary/Conclusions. It is known that DLK1 gene is overexpressed in patients with MDS. A total of 16 samples were studied and 6 (57.5%) were found to be abnormal. It is probable that LOI through epigenetic modifications in the DMR of the GTL2 gene represents a pivotal therapeutic target in MDS. Follow-up for these preliminary results and the study is ongoing. We are going to analyze DNA from a larger number of patients in order to verify our preliminary findings and to study further the imprinting status of the gene.

**Figure 1. Lymphocytes gating of MDS/MPD BM sample.**

**1346**

**COMPARISON OF IN VITRO GROWTH OF GRANULOCYTE-MACROPHAGE COLONY (CFU-GM) FORMATION IN PATIENTS WITH MYELODYSPLASTIC/ MYELOPROLIFERATIVE DISORDERS (MDS/MPD), TYPICAL MYELODYSPLASTIC SYNDROMES (MDS) AND MYELOPROLIFERATIVE DISORDERS (MPD)**

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Myelodysplastic/myeloproliferative diseases (MDS/MPD) are a new category of disorders, which is separated by WHO classification. This group consist of 4 type of disorders: Chronic Myelomonocytic Leukaemia (CML), atypical Chronic Myelogenous Leukaemia (CML), Juvenile Myelomonocytic Leukaemia (JML) and Chronic Myelodysplastic/myeloproliferative disease-unclassifiable (MDS/MPD-U). We still lack understanding of disturbances of hematopoiesis in patients (pts) with MDS/MPD. The objective of the present study was to examine hematopoiesis in pts with MDS/MPD compared with pts with typical MDS and MPD in vitro. We enrolled 96 pts 89 pts with MDS (RA-4, RARS-2, RCMD-17, RCMD/RS-7, 5q-/-, RAEB1-14, RAEB2-7, MDS-U-3), 12 pts with MPD (CML-5, OM-4, CMPD-2) and 25 pts with MDS/MPD (aCML-6, CMML1-8, CMML2-6, MDS/MPD-U-2). Human CFU-GM cells were cultured by plating 1 x 100 mononuclear cells to semisolid methylcellulose medium without or with cytokines (GM-CSF or G-CSF or SCF+GM-CSF+IL-3+Epo). CFU-GM colonies were scored at day 14. We compared spontaneous growth of CFU-GM and in presence of cytokines between patients with MDS/MPD, MDS and MPD. All of the results have been statistically tested by using T-student test for the independent groups. For statistically significant results we were p=0.05. In pts with MDS/MPD according to pts with typical MDS: the spontaneous growth (respectively: med. 2 vs 0; p=0,0042), the growth with G-CSF (respectively: med. 13 vs 3; p=0.0016) and the growth with GM-CSF (respectively: med. 83 vs 16.5; p=0.042) of CFU-GM were statistically significantly higher. In pts with MDS/MPD according to pts with MPD: the spontaneous growth (respectively: med. 2 vs 76; p=0.0005), the growth with G-CSF (respectively: med. 13 vs 146; p=0.0042), with GM-CSF (respectively: med. 83 vs 319; p=0.010) and with GM-CSF+IL-3+Epo (respectively: med. 68 vs 224; p=0.010) of CFU-GM were statistically significantly lower. The growth of CFU-GM in pts with CMML1 was statistically significantly lower according to pts with CMML2 in culture with G-CSF (respectively: med. 5 vs 184.5; p=0.018) and with GM-CSF (respectively: med. 17.5 vs 341.5; p=0.023). Statistically significant differences in culture of CFU-GM between pts with MDS/MPD, typical MDS and MPD verify distinct biology of MDS/MPD. Statistically sig-
ificant differences in growth of CFU-GM in culture with G-CSF and with GM-CSF between pts with CMML1 and CMML2 show another biology isolated by WHO classification subtypes of CMML.

1347 BLAST CELL COUNT IN THE BONE MARROW OF PATIENTS WITH MYELODYSPLASTIC SYNDROME (MDS) OR SECONDARY ACUTE MYELOID LEUKEMIA (sAML): COMPARISON BETWEEN MORPHOLOGIC ASSESSMENT ON MARROW ASPIRATE (AS) AND IMMUNOHISTOCHEMISTRY ON BONE MARROW BIOPSY (BMB)

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Backgrounds. In the French-American-British (FAB) co-operative group and the WHO classification MDS are stratified accordingly to marrow blasts percentage. Blast cell count is also comprised within the international prognostic score system (IPSS), which enables to define four groups with distinct prognosis. Diagnosis of sAML is defined by marrow blast count >30% (FAB) or >20% (WHO), along with previous diagnosis of MDS or dysplastic features of marrow myeloid lineages. Accordingly, precise quantitation of marrow blasts is critical both for diagnosis and prognosis of pts with MDS. AS is currently retained the best tool to assess hematopoietic cellular morphology, actually, quantitation of blasts is afforded by BMB when AS is not available (e.g. dry tap). Aim: to compare marrow blasts percentage quantified by morphology alone on AS and CD34+ blasts by immunohistochemistry on BMB in MDS and sAML patients. Methods. We reviewed the marrow aspirate and core biopsy reports of 169 pts with MDS or sAML at diagnosis, period 1997-2005. Marrow blasts have been morphologically quantified on May-Grunwald Giemsa stained AS and expressed as blast percentage over 500 nucleated marrow cells. Bouin's fixed, paraffin-embedded BMB have been evaluated for CD34+ immature cells counted over 1000 nucleated marrow cells. Diagnoses were assigned a class and compared. FAB, WHO and IPSS, marrow blasts percentages have been grouped into RA=106, RAEB=34, RAEB-T=12, CMML=5, sAML=12. Conclusions. According to morphology of AS and the WHO classification MDS are stratified accordingly to marrow blasts percentage. Blast cell count is also comprised within the international prognostic score system (IPSS). Blast cell count was higher and in the remaining 5 lower on BMB when compared to AS.

1348 BCR-ABL/ABL RATIO AND FISH PH POSITIVITY - RELATION TO C-KIT EXPRESSION AND DUAL ESTERASE ACTIVITY IN CML PATIENTS ON GLEEVEC THERAPY

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Aim: to analyze CD117 expression and dual esterase activity in CML patients on Gleevec therapy. To correlate the results with percentage of FISH Ph positive HCs and bcr/abl/abl ratio. Methods. 22 bone marrow specimens of 17 CML patients on Gleevec therapy (duration of therapy 4 - 32 months) were analyzed by FISH and quantitative RT-PCR, immunocytochemical APAAP CD117 expression and cytochemical dual esterase activity. Patients were divided in subgroups according to duration of therapy (less than 6, 6-12 and more than 12 months). Results. Patients with leukocyte differential count changes had high bcr/abl/abl ratio, FISH Ph and CD117 positive HCs. Medians of CD117 and FISH Ph positive HCs were highest in CML patients during the first 6 months of Gleevec treatment, but correlation of these two parameters was low (0.19). There was no statistical difference when medians of CD117 and dual esterase positive HCs were compared between subgroups. Correlation of FISH Ph positive HCs and bcr/abl/abl ratio was high (0.68) with constant decrease in percentage of FISH Ph positive HCs and bcr/abl/abl ratio during follow-up. Conclusions. According to low correlation obtained between CD117 expression and dual esterase activity with percentage of FISH Ph positive HCs and bcr/abl/abl ratio, FISH and quantitative RT-PCR are the methods of choice for monitoring the efficiency of Gleevec therapy.

1349 THE EFFECT OF HYPERTERMIA ON DIFFERENTIATION INDUCTION AND APOPTOSIS OF K562 ERYTHROLEUKEMIC CELL LINE: RELATIONSHIP WITH HEAT SHOCK PROTEIN 70 (HSP70)

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Hyperthermia causes a variety of morphological and functional effects in various cancer cells. At high temperatures, hyperthermia can induce differentiation in different tumor cells including leukemia cells, but severe treatments cause cell death by apoptosis or necrosis. Hyperthermia also effects on heat shock protein gene expression. Since heat shock protein 70 (HSP70) has a crucial role in cell differentiation and cytoprotection, this protein may have a role in differentiation and apoptosis induced by hyperthermia in K562 erythroleukemia cells. In the present work we have studied the effects of mild and severe treatments on differentiation induction and apoptosis in K562 cells. For this purpose, differentiation and apoptosis were measured along with the level of HSP70 protein. Erythroid differentiation was measured by benzidine staining assay and analyzing the expression of glycoporphin A by flow cytomtery technique. Apoptosis was evaluated by flow cytomtery method based on binding of Annexin V and DNA staining by PI. DNA fragmentation was also studied. HSP70 protein level was determined by HSP70 ELIZA kit. Our results showed that mild hyperthermia (43°C) reduced cell growth and induced differentiation without affecting cell viability but heating cells at 45°C reduced the viability and totally inhibited the growth of these cells and no sign of differentiation was observed. On the other hand, mild hyperthermia (43°C) had not significant effect on induction of apoptosis in these cells, while, 45°C temperature caused cell death by apoptosis and necrosis. The level of HSP70 protein increased in cells treated with 45°C compared to the control cells, while, no significant increase could be detected at 48°C. In conclusion, increase in HSP70 protein level in 43°C heated cells can cause cytoprotection and lead to their differentiation, while, severe treatment, which cause no increase in HSP70 protein level, may lead to apoptosis of these cells.

1350 MUCOSITIS IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA (CML) ON IMatinib, AN INDIAN EXPERIENCE

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To evaluate the incidence and severity of mucositis in patients with chronic myeloid leukemia (CML) on Imatinib. Retrospective data analysis conducted at Kidwai Memorial Institute of Oncology, Bangalore, India, a tertiary care cancer center, with an annual attendance of 16000 new cases. All patients of CML who were on Imatinib were analysed. They were stratified into chronic phase (CP), accelerated phase (AP), and blast crisis (BC). The CTC criteria was used to assess mucositis. A total of 210 patients with complete clinical data were analysed. Details are shown in Table 1.
The majority of patients (90%) in BC developed mucositis, while AP (60%), and CF (26%) had a lower incidence. Mucositis onset was within the first 3 months of initiating imatinib in the majority (87%) of the patients (p value <0.01), however, no patient required dose reduction or cessation of therapy due to mucositis. The median time for resolution of mucositis was 6 weeks irrespective of the stage of CML.

**1351**
IDENTIFICATION OF A RARE E6A2 BCR-ABL FUSION TRANSCRIPT IN CHRONIC MYELOID LEUKEMIA: A CASE REPORT

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The balanced translocation t(9;22)(q34;q11) producing the chimeric BCR-ABL transcript is the hallmark of chronic myeloid leukemia (CML). Commonly, the breakpoints in the BCR gene occur within the major breakpoint cluster region (M-bcr), producing different types of BCR-ABL transcripts (e1a2 and/or e14a2). Breakpoints outside M-bcr occur rarely, in either minor-bcr (m-bcr) or micro-bcr (µ-bcr) leading to e1a2 and e19a2 fusion transcripts, respectively. Atypical BCR breakpoints outside these cluster regions are very rare. In particular, five cases of Philadelphia (Ph) positive CML and one case of CMML in progression have been associated with an atypical e6a2 BCR-ABL fusion transcript. The breakpoint in bcr intron 6 has been implicated as the cause of a more aggressive clinical phenotype and of increased oncogenic potential, due to the partial loss of important regulatory BCR sequences. The aim of this study was to examine the relationship between BCR-ABL and CCN3 in primary human cells and to determine if CCN3 expression could provide an additional marker of Ph+ CML. The presence of this transcript was confirmed in cell line models that the posse of the growth regulator, CCN3, is downregulated as a result of Bcr-Ab1 kinase activity and that CCN3 has a reciprocal relationship with expression of BCR-ABL. The aim of this study was to examine the relationship between BCR-ABL and CCN3 in primary human cells and to determine if CCN3 expression could provide an additional marker of Ph+ CML. The presence of this transcript was confirmed in cell line models that the positive of the growth regulator, CCN3, is downregulated as a result of Bcr-Ab1 kinase activity and that CCN3 has a reciprocal relationship with expression of BCR-ABL. The aim of this study was to examine the relationship between BCR-ABL and CCN3 in primary human cells and to determine if CCN3 expression could provide an additional marker of Ph+ CML.

**1352**
CCN3 EXPRESSION MAY PROVIDE AN ADDITIONAL MARKER OF RESPONSE TO IMATINIB

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Chronic Myeloid Leukemia (CML) is characterized by expression of the constitutively active Bcr-Abl tyrosine kinase. Molecular monitoring of BCR-ABL expression levels by Real-time PCR allows profiling of minimal residual disease for patients being treated with imatinib. We have shown previously in cell line models that the negated growth regulator, CCN3, is downregulated as a result of Bcr-Ab1 kinase activity and that CCN3 has a reciprocal relationship with expression of BCR-ABL. The aim of this study was to examine the relationship between BCR-ABL and CCN3 in primary human cells and to determine if CCN3 expression could provide an additional marker of Ph+ CML. The presence of this transcript was confirmed in cell line models that the positive of the growth regulator, CCN3, is downregulated as a result of Bcr-Ab1 kinase activity and that CCN3 has a reciprocal relationship with expression of BCR-ABL. The aim of this study was to examine the relationship between BCR-ABL and CCN3 in primary human cells and to determine if CCN3 expression could provide an additional marker of Ph+ CML. The presence of this transcript was confirmed in cell line models that the positive of the growth regulator, CCN3, is downregulated as a result of Bcr-Ab1 kinase activity and that CCN3 has a reciprocal relationship with expression of BCR-ABL. The aim of this study was to examine the relationship between BCR-ABL and CCN3 in primary human cells and to determine if CCN3 expression could provide an additional marker of Ph+ CML.
induced 7-65% growth inhibition of NB4 cells. Cell viability was also decreased by 2-55% between 24 to 96 h treatments with the drug. These effects of the drug were also dose-dependent. 3-HK induced a significant G1-arrest up to 48 h which consequently followed with appearance of sub-G1 peak (apoptosis) at 72 and 96 h. In addition we confirmed that the inhibition of proliferation is associated with differentiation especially toward macrophage-like morphology. Conclusion. We showed that 3-HK is a potent differentiating and apoptotic agents. These results can introduce 3-HK as potent candidate to treatment of leukemia.

1354
THE ROLE OF MDR RELATED PROTEINS IN THE PROGNOSIS OF ADULT ACUTE MYELOID LEUKEMIA (AML) WITH NORMAL KARYOTYPE
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Background. Cytogenetic abnormalities are among the most important factors affecting the outcome of patients with acute myeloid leukemia (AML), but approximately 40-50% of AML cases display a normal karyotype at diagnosis. In the last years the over-expression of MDR related proteins has emerged as a factor negatively affecting outcome in leukemia patients, especially in cases with abnormal karyotype. Less defined is the impact of drug-transporter proteins in AML with normal diploid cytogenetics. Aims. We have compared the expression of P-glycoprotein (PGE), multidrug-resistance related protein (MRP) and lung resistance protein (LRP) with the clinical and biological characteristics of 135 adult patients with normal karyotype AML, to evaluate their possible impact on response to therapy and on survival. Methods. Median age was 55 years and 60 out of 135 (44%) patients were older than 55 years. Therapy consisted of a standard 3/7 (idarubicin and cytarabine) course as induction and intermediate-dose cytarabine (2 courses) as consolidation for 38 patients. Fludarabine-based induction course (FLAI / FLAIE) followed by intermediate-dose cytarabine as consolidation was used in 65 patients. Thirty-two patients were included in a clinical trial comparing FLAI to ICE as induction course, followed by a consolidation course of high-dose cytarabine. For statistical analysis response to therapy was evaluated after two chemotherapy courses. Patients who underwent allogeneic stem cell transplantation were censored at time of transplant.

Results. Increased PGP expression was associated only to advanced age (p=0.003). Conversely, no difference in the two age cohorts was found in MRP and LRP expression. No association was assessed between PGP, MRP and LRP over-expression and clinical and biological characteristics. Complete remission was strongly affected by PGP over-expression. In fact only 13/34 (15%) PGP-negative, but 19/48 (44%) PGP-positive patients did not respond to chemotherapy (p = 0.006). Advanced age and CD34 positvity on blast cells confirmed their negative role on obtainment of remission. No impact on response to therapy was demonstrated for MRP and LRP. However, a lower percentage of complete responses was observed in those patients over-expressing more than one MDR protein (p=0.03). Event-free survival of the whole population was 9 months. In the univariate analysis EFS was influenced by PGP over-expression (10 vs 4 months, p=0.035). EFS was negatively affected also by age (p>0.0008) and CD56 (p=0.044). In multivariate analysis all this factors retained their statistical significance. Summary/Conclusions. In our study only PGP expression showed a negative correlation with response to induction therapy, as well on EFS. MRP or LRP did not influence treatment outcome when singularly considered, but patients over-expressing more than one MDR-related protein had a lower probability to achieve CR. Age was the most important factor affecting EFS, but shorter EFS duration was observed also in PGP-positive patients and in those with CD56 aberrant expression. Our data confirmed the prognostic role of MDR proteins also in the subset of AML patients with normal karyotype, and could be used to stratify patients with different prognosis and to design risk-adapted therapeutic strategies.

1355
THE EVALUATION OF ANTIMURAL AND DIFFERENTIATION OF PLANT-DERIVED AGENTS IN COMBINATION WITH ATRA ON LEUKEMIC CELLS
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Backgrounds. Acute leukaemia is characterised by accumulation of neo-plastic cells which fail to develop into mature cells. Cytotoxic, differenti- ation and apoptotic agents have been employed for treatment of leukaemia. In iranian traditional medicine plant-derived agents have been used for treatment of cancer. Aims. The present study is evaluation of cytotoxicity, apoptosis and differentiation of several plant extracts such as Peganum Harmala, Harmine, Harmaline, Urtica Dioica, Chelidonium Majus and Viscum Album on HL60 cells. ATRA has been used as standard agent. However, little study have been reported using these agents on this cell line, these components were initiated such as an investig- ation. Methods. HL60 cells were cultured to cells and incubated for 5 days. Counting of cells, viability, MTV, morphology, NBT reduction and cytofluorometric analysis performed by FACS using PI for cell cycle ,markers including CD11b and CD14 for myeloid differentiation and apoptosis using Annexin V. Results. The data showed that all agents in optimal dose caused cessation of proliferation in dose and time dependent manner(p<0.05). Optimal concentration of Peganum Harmala, Harmine, Harmaline, Urtica Dioica, Chelidonium Majus and Viscum Album (10 µg/ml,1.6 µg/ml,10 µg/ml, 2.5 mg/ml, 0.1 mg/ml and 50 µg/ml respectively) were chosen as antiproliferative effect with good viability. However, all agents in higher concentration were toxic. Treated cells with ATRA showed depletion of growth in optimal dose of 10-7 Mol. Cells accumulated in G1 phase using ATRA (81.5%), Urtica Dioica (75%) and Viscum Album (72%) but they arrest- ed in S phase using Peganum Harmala, Harmine, Harmaline (52.7%) and Chelidonium Majus (54.5%). Only, cells induced by Harmaline 10 micg/ml showed myeloid differentiation with some morphological changes , NBT positivity (28%) and increase in CD11b (24.3%) and CD14 (43.5%) (p<0.05) compared to ATRA (40% as NBT, 71% and 5.7% as CD11b and CD14).However, Viscum Album showed some apoptotic changes in 100 micg/ml concentration. The combination of these agents in optimal dose with ATRA did not show any effect on differ- entiation of cells and ATRA preserved effect of differentiation of itself with higher cessation of proliferation. Conclusions. In conclusion , these data showed that the combination of these plant extracts with cytotox- ic and differentiation agents may open a new window in leukemic in vitro therapy which requires further investigation.

1356
NO INFLUENCE OF A POLYMORPHISM IN ENDOSTATIN, AN ANGIGENESIS INHIBITOR, FOR THE RISK OF ACUTE MYELOID LEUKEMIA
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Backgrounds. Angiogenesis is an important step in solid tumours and leukaemias development and progression. In addition to producing proangiogenesis cytokines, there is evidence that neoplastic cells also play a role in the generation of extracellular matrix remodelling, such as endostatin (ES). ES is a 20kDa C-terminal fragment of collagen XVI- II, the product of the COL18A1 gene. Higher serum levels of ES induced experimentally in mice caused regression of leukaemia and solid tumours. In addition, Down’s syndrome patients have a decreased inci- dence of solid tumours possibly due to the high serum levels of the protein produced by their three copies of the COL18A1 gene. Thus, differ- ent levels of ES seem to be associated with varying susceptibility to tumour development. Furthermore, a COL18A1 gene polymorphism (D104N) located in the COOH-terminal globular domain, NC1, of col- lagen XVIII, the encoding region for ES was recently associated with increased risk for the prostatic adenocarcinoma, which was attributed to an impairment in the protein function. Aims. In this study, we tested whether D104N polymorphism of the COL18A1 gene alters the risk for AML. Methods. Genomic DNA from peripheral blood of 122 AML patients (74 men, 48 women; mean age±SD: 46.8±17.9 years; 106 Caucasians, 16 Blacks), seen at the University Hospital of the State University of Campinas, and 351 controls (198 men, 153 women; mean age±SD: 52.9±4.5 years; 302 Caucasians, 49 Blacks), seen at the University Hospital of the State University of Campinas, were analysed using the polymerase chain reaction (PCR) followed by restriction endonuclease digestion with Msp I. Results. Both the patients’ and controls’ samples were in Hardy-Weinberg equilibrium (X² = 0.77, p=0.397; X² = 1.99, p=0.17,
for heterozygous D104N genotypes, respectively). We have observed similar frequencies of D104N genotypes in AML patients and controls (14.8% and 13.7%, respectively; p=0.76). Similar risks for the disease were also seen in individuals with heterozygous D104N polymorphism in comparison with the wild genotype (OR= 1.09, 95%CI: 0.61-1.96).

Considering only the AML patients, no differences in the frequencies of D104N polymorphism were found according to gender (12.2% in male vs. 18.8% in female; p=0.44), age (19.6% in 75 patients under 50 years vs. 22.5% in 49 patients at an older age; p=0.07), and ethnic origin (15.1% in Caucasian patients vs. 12.5% in Black patients; p=1.00). Conclusion: Our results present preliminary evidence that the D104N polymorphism of the COL1A1 gene may be an unimportant determinant of the AML susceptibility.

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1357 OUTCOME OF ALLOGENIC STEM CELL TRANSPLANTATION IN ADULT PATIENTS WITH PH+ AML


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Backgrounds: The Philadelphia chromosome positive (Ph+) acute myeloid leukemia (AML) is rarely found in adult patients with an overall incidence of less than 1%. Most patients with Ph+ AML have an extremely poor prognosis when treated by chemotherapy alone. Recently, allogeneic hematopoietic stem cell transplantation (HSCT) performed early during remission with an improved treatment agent, using an inter- therapy of imatinib (Glivec, STI571), has suggested a long-term survival. As a result, we analyzed a future treatment strategy, we analyzed the effect of imatinib addition into standard chemotherapy as an alternative before allogenic HSCT for newly diagnosed Ph+ AML patients in first CR. To better understand a role of imatinib in adult Ph+ AML and chemotherapy followed by autologous peripheral blood stem cell transplantation (auto-PBSCT) as post remission therapy for intermediate-risk AML patients in first complete remission (CR) has been reported to have encouraging results. We adopted this approach at our institution. In the 5-year period from 1999 to 2003, 17 patients who satisfied these criteria underwent auto-PBSCT. They comprised 12 male and 5 females with ages ranging from 15-66 years (median - 48years). The aim of our study was to retrospectively evaluate the outcome of a unique treatment modality with a low relapse rate. AIMS: To assess the impact of post-remission PBSCT in patients with newly diagnosed Ph+ AML.

Methods: Between Nov 2001 and Oct 2004, 12 (2.2%) of the 556 adults (age, 17~84) with AML were Ph+ at the time of diagnosis. Of these, 5 patients with newly diagnosed Ph+ AML who completed induction chemotherapy and received matched or mismatched allogeneic HSCT were investigated in this study. Overall complete remission rate was 58.3% (7/12) by intention-to-treat analysis. Two patients were excluded because of their refusal of study. The median follow-up period for all 17 patients ranged from 6-45.5 months (median - 25months). No patients were lost to follow up. The 3-year survival was 55.6% for FLT3 ITD positive compared with 33.3% for the negative cases. Overall survival (OS) was significantly better for those without FLT3 ITD mutations (p=0.05, log rank test). By Kaplan-Meier curve, FLT3 positive patients had a higher relapse rate. However the trend towards shorter disease free survival (DFS) for the FLT3 ITD positive (37.5%) versus negative (55.6%) cases at 3 years could not be demonstrated to be statistically significant (p=0.1709), possibly due to small sample size. In summary, determination of status of FLT3 ITD mutations at diagnosis is important in risk stratification management of patients with normal karyotype AML in first CR. Unlike the experience of Yoshimoto et al who found that myeloablative chemotherapy supported by auto-PBSCT in such patients may overcome the poor prognostic implications of FLT3 mutations, we have not found this to be so. Since 2004, we have amended our approach to offer allogeneic bone marrow transplantation upfront to FLT3 ITD positive AML patients in first CR where possible.

1359 PROGNOSTIC SIGNIFICANCE OF FLT3 MUTATIONS IN DIFFERENT SUBTYPES OF ACUTE MYELOID LEUKEMIA

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Backgrounds: Fms-like tyrosine kinase 3 (FLT3) is a member of the class III receptor tyrosine kinase family along with KIT, FMS and platelet derived growth factor receptor. Wild type FLT3 is expressed at high levels on 70% to 100% of blasts in acute myeloid leukemia (AML). FLT3 gene alterations, internal tandem duplications (ITDs) and Asparaginase (D555) mutations occur in 15%-30% in AML and may adversely affect clinical outcome. AIMS: The aim of our study was to analyze the impact of FLT3 mutations in cohort of 113 newly diagnosed patients with AML on prognosis. Methods: Genomic DNA polymerase chain reaction (PCR) assay was performed to detect FLT3/ITD located from exon 14 to exon 15 and we used PCR-restriction fragment length polymorphism (PCR-RFLP) for detection of D555 mutations in exon 20. Results: FLT3/ITD were detected in 20/113 patients (pts) (17.6%). D555 mutations in 4/113 (3.5%) and both type of mutations in 1 (0.8%) pt. In the study group of 113 pts according to FAB classification, FLT3 mutations were found in all subtypes except M1, M6 and M7. The distribution of FLT3 mutations was as follows: FLT3/ITD was detected in M0 6/12 (50%) pts, 4/22 (16.7%) in M2, 3/14 (21.42%) M4, 3/24 (12.50%) M4, and 8/19 M5 pts (40.3%). D555 mutation was found in 1 pt with M2 and M5 and 2 pts with M4 type of AML. Of 24 pts with FLT3 mutations a normal karyotype was found in 9 pts, 3 pts had translocation (15;17), 2 pts had inv (16), 1 in 19. Three pts had complex karyotype, and in 2 pts there were no mitoses on preparation. Treatment included induction chemotherapy with doxorubicin 90 mg/m^2 3 days and ara-c 200 mg/m^2 in continuous infusion for 7 days. Consolidation therapy consisted of the same scheme or ADE combination. Complete remission in the whole cohort of patients was achieved in 62% and in
only 7/24 (29%) pts with FLT3 mutations (one patient with M0 and normal karyotype, one with M5a and also normal karyotype, 3 pts with M3 and translocation (15; 17), one patient with M2 and deletion 19, one with M4 and inv(16)). FLT3/ITD+ pts had significantly higher WBC count at diagnosis (WBC count for FLT3/ITD+ was 73.5±10/L and WBC count for FLT3/ITD- was 14.9±10/L, p<0.05). Median of overall survival of the whole group of pts was 11 months, and median of survival of pts with FLT3 mutations was 6 months. Conclusion: In contrast to other reports incidence of FLT3/ITD and D835 mutations are lower in our cohort although the study group was small and performed in a single Institution. With a median follow up of 46 months remission duration and overall survival were significantly shorter for patients with FLT3 mutations.

1360 MONITORING OF CARDIOTOXICITY DURING INDUCTION CHEMOTHERAPY CONTAINING IDARUBICIN IN ACUTE MYELOID LEUKEMIA WITH CIRCULATING MARKERS

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Backgrounds. Cardiac toxicity is a well-known and serious complication of antitumourous treatment. Anthracyclines represent the greatest risk for cardiotoxicity. We have evaluated the incidence of structural and functional myocardial damage have been gaining ground in cardiotoxicity diagnostics. Aims. Monitoring of cardiotoxicity during induction chemotherapy in acute myeloid leukemia (AML) patients and assessment of the potential for use of circulating markers in early diagnostics of cardiotoxicity. Methods. Fifteen consecutive adult patients with a newly diagnosed AML (9 male and 6 female, mean age 43.7±10.6 years) participated in the study. The patients received induction chemotherapy containing intermediate doses of cytarabine and idarubicin (IDA) 12 mg/m2/day intravenously on day 1, 3 and 5 (in total 36 mg/m2 = 1/4 of a newly diagnosed AML (9 male and 6 female, mean age 43.7±10.6 years) of cardiotoxicity. 3. may lead to congestive heart failure and NT-proBNP, which is to determine the level of TPO in AML patients in order to characterize its possible impact on AML prognosis remains to be characterized. Aim. Is to

1362 PROGNOSTIC RELEVANCE OF SOLUBLE TPO LEVEL IN AML

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Backgrounds. Thrombopoietin (TPO), the major growth factor for cells of the megakaryocytic lineage is removed from circulation by binding to c-mpl receptors present on platelets and megakaryocytes. Recently functional c-mpl receptors was reported on the AML blast cells and its clinical impact on AML prognosis remains to be characterized. Aims. To determine the level of TPO in AML patients in order to characterize its clinical relevance. Methods. We assessed TPO levels by ELISA in 41 AML patients at diagnosis, after 28 days of induction chemotherapy and at AML remission. Follow up for the patients was done up to 24 months. Results. TPO levels was significantly higher at diagnosis as compared to normal controls (p<0.01). At 28 days after induction chemotherapy the TPO level continue to elevate and was significantly higher as compared to the diagnosis level (p<0.01), and then decline during remission reaching near the control level (p>0.05). The TPO levels was inversely correlated to the platelets counts (R=-0.9, p<0.01). TPO level at AML diagnosis was significantly lower in a group of patients who died during the follow up course(n= 25) and in patients resist induction chemotherapy (n=8) as compared to patients who survive and patients who respond to chemotherapy (p<0.05 for both).

1363 IMPACT OF ADDITIONAL CYTOGENETIC ABNORMALITIES ON REMISSION INDUCTION RATE EVENT FREE AND OVERALL SURVIVAL IN 34 PATIENTS WITH NEWLY DIAGNOSED ACUTE PROMYELOCYTIC LEUKEMIA TREATED WITH APL393 TUNISIAN EXPERIENCE

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Additional chromosomal abnormalities in acute promyelocytic
leukemia (AML) are observed in around 30% of cases. Their presence seems to have a mild impact on prognosis. In our study it is to analyze impact of additional cytogenetic abnormalities on complete remission rate (CR) event free survival (EFS) and overall survival (OS) in 34 consecutive patients with AML and t(15,17) treated with AML93 protocol between 1998 and 2004. Median age was 28 yr (6-60 yr). Median WBC was 5000/mm³ (600-9700/mm³). Informative karyotype was obtained in 32 patients. Additional cytogenetic abnormalities were seen in 9 patients 26.47% (9/34). These abnormalities were +8q(4),add 9q(1),del 9q12;3q(11); der t(17q(1),add 15q(1), add 5p(15). For all patients CR was 82% (28/34), failure of induction was due to 6 toxic deaths: sepsis (1), ATRA syndrome (2), SNC hemorrhage (2), and diabetes (1). EFS at 4 yr is 63, 47% and OS at 4 yr is 69, 72%. Outcome was similar between patients with t(15,17) alone and patients with additional cytogenetic abnormalities for CRR 84% (21/25) vs 77.7% (9/12) p=0.6, for EFS at 4 yr 62.02% vs 66.67% p=0.74 and for OS at 4 yr 64.81% vs 70.82% p=0.5. Our study does not find any significant impact of additional abnormalities despite a little advantage for EFS and OS for this group.

1364 HUMORAL IMMUNE RESPONSE AGAINST THE PRAME ANTIGEN IN PATIENTS WITH MYELOID LEUKEMIAS
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Backgrounds. The PRAME (preferentially expressed antigen in melanoma) is expressed at high levels in various malignant tumors including hematopoietic malignancies, especially in acute myeloid and lymphoid leukemias (AML, ALL), multiple myeloma etc. It has no or weak expression in normal tissues making it a candidate for immunotherapy. FRAME can also elicit T-cell immune response in melanoma patients but there are no data concerning anti-FRAME immune response in leukemias. Aims. To detect specific immune response towards FRAME in patients with myeloid leukemias (AML, CML). Methods. Sera obtained from patients with myeloid leukemias were analyzed in enzyme-linked immunosorbent assay (ELISA) for detection anti-FRAME antibodies. Results. IgG FRAME antibodies were measured in 122 patients (25 AML, 97 CML) and 22 healthy volunteers. Immunoglobulin IgG FRAME antibodies were detected in 4 (16%) and 8 (8%), respectively, of 122 patients, whereas none of the healthy volunteers had IgG FRAME antibodies. In one of IgG FRAME-positive AML samples the specific cytotoxic T-lymphocytes were identified by MHC-peptide tetramer staining with intracellular interferon-γ co-staining. Summary: The data demonstrate that spontaneous humoral immune responses against FRAME protein could be detected in the patients with FRAME-expressing hematopoietic malignancies.

1365 IS LEUKAPHERESIS ABLE TO IMPROVE SURVIVAL IN HYPERLEUKOCYTIC AML IF USED AS THE EARLY CYTOREDUCTION TREATMENT?
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Background. The management of patients with AML presenting with hyperleukocytosis remains controversial. In spite of relatively high incidence of hyperleukocytosis (7-15%) and its very high early mortality (>40%), there is no consensus in the optimal treatment for a prompt leukoreduction. Aims. The aim of this retrospective non-randomized study was to compare early mortality and overall survival (OS) in patients with hyperleukocytic AML initially treated with either hydroxyurea (HU) alone or HU and leucapheresis. Patients and Methods. From 1998 to 2005, 40 patients were treated for hyperleukocytic AML (MO=22, M1=8, M2=7, M3=2, M4=16, M5=5) in our institution. Group A consisted of 20 patients with median age of 67 years (19-78) treated by HU only (50 mg/kg/day in 3 or 4 daily doses). Group B consisted of 20 patients with median age of 53 years (19-72) treated with HU and cytoreduction leukapheresis. The intention of the cytoreduction treatment was to decrease WBC from at least 500x10⁹/L before administration of an induction chemotherapy to prevent complications from leukostasis and tumor-lysis syndrome. Leukaphereses were performed using COBE Spectra cell separator. Results. The early mortality was high according to the expectations: seven patients died within two weeks in group A, as well as in group B. The patients from the group B were generally in worse condition and 4 of them died within the first 48 hours for intracranial hemorrhage or respiratory failure (ARDS) because of leukostasis. The target cytoreduction in the group A was delayed compared to the group B, although the initial WBC count was lower (160x10⁹/L vs. 200x10⁹/L, means). In the group B, forty leukaphereses were performed in total, median 2 (1-4) per patient. Induction chemotherapies could start earlier in the group B compared to group A: on day 4 (median, range 2-12), median WBC count 30.2x10⁹/L and on day 1 (median, range 1-5), median WBC 24x10⁹/L. Thirty induction chemotherapies were administered in total, 14 in the group A and 16 in the group B. One patient from the group B refused chemotherapy and died of leukemia in 11 days. Complete remissions were reached in 15 patients, but only in 5 from the group A. OS was significantly longer in the leukapheresis group (p<0.05), however, we did not confirm improvement of the 2-week mortality. Median OS in the group A was 30 days and no patient survived more than 500 days. Median OS in the group B is 282 days and 6 patients are still alive from 2 to 5.7 years after the AML diagnosis. Summary/conclusions. Current published data do not define the impact of using leukapheresis for the cytoreduction before the individual response in a clinically relevant time, we analysed the clearance of peripheral blasts (PBC) in 30 AML patients during “3+7” induction course. Methods. By extensive flow cytometry (FC), a population of cells with leukaemia-associated aberrant immuno-phenotype (LAIP) was identified in each patient from the initial bone marrow (BM) aspirate. We then obtained the absolute counts on peripheral blood (PB) immediately before starting therapy (day 1) and every day until day 8. PB was expressed as the ratio, converted to logarithmic scale, between baseline value (day 1) and daily absolute blasts count. At day 14, FC analysis was performed on BM in order to identify LAIP-positive residual blasts. The degree of BM clearance was expressed as the ratio, converted to logarithmic scale, between the percentage of LAIP-positive blasts determined at diagnosis and day 14 (LD14). Results. Between May 2004 and January 2006, 30 consecutive newly diagnosed non-M3 AML patients aged less than 66 years entered the study and were evaluable for BM response. After a single course, complete remission (CR) was achieved in 17 patients. CR was not obtained in 13 patients (NCR), 8 of whom were refractory. According to conventional criteria (cytogenetics and secondariness) there were 11 high risk patients, of whom 4 achieved CR; 14 intermediate risk patients, of whom 8 achieved CR; 5 low risk patients, all of whom achieved CR. The ranges of distribution of PBC had minimal overlap between CR and NCR groups. Since in patients who achieved CR, by day 7 or 8 blasts were often already undetectable, we excluded these time-points from analysis (Figure 1A). The medians of log reduction in the two groups were significantly different on each day (Figure 1B). The rate of PBC appeared higher in CR than NCR patients with an estimated difference between groups equal to 0.26 (95% CI 0.15-0.37, p value<0.001). This difference was not attributable to differences in baseline PB leukemic burden and assigned risk. PBC showed an excellent correlation with BM response as assessed by morphologic analysis at haematopoietic recovery and by FC on day 14. Specifically CR was not achieved in any of 11 patients who had a PBC below 2 logs on day 5, whereas CR took place in 17 out of 19 patients who had a PBC greater than 2 logs on day 5. Higher values of PBC on each day were associated with larger LD14 (Figure 1C). This correlation was significant on each day and it increased monotonically over days. Summary/conclusions.
These data indicate that PB may be in equilibrium with BM in each AML patient, and that PB clearance gives evidence of BM clearance. Therefore, a major treatment outcome may be predicted very early during the induction therapy of AML patients, thus providing an opportunity to tailor treatment modalities since the outset.

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CLINICAL SIGNIFICANCE OF MULTIDRUG RESISTANCE 1 (MDR1) GENE EXPRESSION FOR TREATMENT OUTCOME IN CHILDHOOD ACUTE LYMPHOBlastic LEUKEMIA

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A major cause for early relapse and treatment failure in patients with acute lymphoblastic leukemia (ALL) is the occurrence of multidrug resistance (MDR). One of the mechanisms is the overexpression of MDR1 gene which encodes a drug efflux pump called permeability-glycoprotein (P-gp). The aim of this prospective study was to analyse the expression of MDR1 gene and correlate our findings with clinical, laboratory parameters and treatment outcome in children with ALL. Material and Methods. We studied prospectively 49 children with ALL (26 boys and 23 girls) with median age of 5.1 years (range: 18 months to 13.9 years). Four children were also evaluated at initial diagnosis and relapse. All patients were treated according to the BFM95 chemotherapy protocol with a median observation time of 18 months (range 9-36 months). As controls we used bone marrow (BM) mononuclear cells from 7 children who underwent a BM biopsy for diagnostic purposes and was negative for leukemia. Total RNA was isolated from BM samples at initial diagnosis and relapse. The expression of MDR1 gene and the housekeeping β-actin gene was detected by RT-PCR using the appropriate primers. After electrophoresis of the PCR products in 1.5% agarose gel stained with ethidium bromide, gels were scanned by UV transillumination with a densitometer. The relative mRNA expression of MDR1 gene was calculated using the following formula:

Expression Index (EI): MDR1 PCR product / β-actin PCR product

Results. The mean MDR1 gene EI was significantly higher compared to the control group (p<0.05). The MDR1 EL in patient sample ranged from 0.02 to 2.49 (median 0.35). Using the median as a cut-off value for high and low expression, high MDR1 EI was found in 18 (36.7%) patients and their event free survival was significantly worse compared with children with low MDR1 expression (86.67% vs. 55.56%; p log-rank: 0.05). High expression of MDR1 gene did not correlate with immunophenotype, NCI risk classification, white blood count, prednisone response on day 8, and LDH value. Interestingly, significantly higher MDR1 EI was found at relapse in four paired samples compared with diagnosis. Cox regression analysis revealed that children with high MDR1 expression at diagnosis had a relative risk of 3.86 (range: 1.02-11.46) for failure to achieve a complete remission or relapse (p=0.04). Conclusion. The expression of the MDR1 gene in childhood ALL is a useful tool in assessing the risk of treatment failure and early relapse and can be used as an additional prognostic factor.

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PERIPHERAL BLOOD B-LYMPHOCYTE SUBSETS AFTER TREATMENT OF CHILDHOOD ACUTE LYMPHOBlastic LEUKEMIA

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The immunosuppressive effect of cytotoxic drugs, basic therapeutic agents in the treatment of childhood acute leukemias, requires monitoring of the immune system following cessation of therapy. The aim of the study was to analyse the examination of the relative and absolute numbers of CD19+, CD20+ and CD5+ B lymphocytes in peripheral blood in children with acute lymphoblastic leukemia (ALL) after cessation of chemotherapy. The examined group included 150 children with standard-risk ALL treated with standard chemotherapy protocol. The analyses were performed directly after therapy in 30 children, in 30 children - 3 months later, in 30 children - 6 months later, in 30 children - 9 months later, and in 30 children - 12 months after therapy cessation. The control group consisted of 30 healthy age-matched children. Lymphocyte populations were analyzed by multiparameter flow cytometry with 3-color analyses. Phenotypes of B-cell subsets were obtained and analyzed with Cell Quest software (Becton Dickinson). The proportion of lymphocytes stained with each monoclonal antibody was determined relative to the absolute number of lymphocytes per microliter by multiplying the absolute number of lymphocytes per...
In treating acute lymphoblast leucosis (ALL) in children in terms of ALL-BFM-90(m) program, methotrexate (MTX) utilized intravenously, 1 g/m², combined with an intrathecal injection penetrates the hema-

toencephalic barrier and harms the brain. Purpose. Disclosure of early adverse brain reactions. A bioelectro brain activity (BEAB) was studied using an electroencephalogram (EEG). Nihon Kohden, Japan. Features of the blood brain supply were investigated utilizing transcranial ultra-

sound dopplerography (TUSDG) Logiq 4, Krönzbuhler, Germany. Quantitative assessment of the EEG values was done through the program of carting the DX-complexes-4000, including spectral analysis on the basis of Fourier transform. The results were statistically processed using software. Statistical data in the groups was carried out by t-criteria of equality of average.

Study included 32 children, aged 8,1 to 13,5. Following the program polychemotherapy ALL-BFM-90(m) they received protocol 1 and achieved remission. Each child was subjected to EEG 12 times: a day before the administration of MTX, following an intravenous injection of 1/10 dose; three hours later; at the end of 3 and 13 days. The linear blood flow rate (LBFR) in the arteries of basis cerebri and the blood flow rate in the direct sinus were studied prior to administration of MTX, a day following the injections and at 5 and 13 days. After injection of 1/10 MTX, negative dynamics of BEAB progressing in 2-3 days was noted in all patients’ occipital lobes, i.e. an increase in delta-range slow waves and acute slow wave complex. Positive dynamics was noted seven days later. A large percentage of all the rhythms was registered in the right hemisphere before injecting MTX, shifting to the left hemisphere after injec-

tion of 1/10 dose. Large representation of rhythms’ in the left hemi-

sphere kept up the following 5 days. An average LBFR was normal before injection, one day later it authentically (p<0,05) decreased on both sides, increased 5 days later, and at day 13 there was still a deficien-

cy of blood flow. Blood flow rate in direct sinus increased authentical-

ly (p<0,05) a day after injecting MTX, which was a result of venous out-

flow impairment from the brain surface along the ponticular veins into the upper sagittal sinus, suggesting the venous outflow passed through the direct sinus of brain and the direct sinus. At day 13 the outflow restored, however, did not return to norm. The EEG analysis revealed a buildup of 6-range slow waves and appearance of the acute slow wave complexes in the occipital regions after an intrathecal injection of MTX, indicating an increase in excitability of the mesodiencephalic structures. The right hemisphere was dominant before and the left after injecting MTX as a result of complectional proceral interaction of the left and right hemispheres and the right hemisphere having damper effect on the left one. The fall of LBFR in the arteries of basis cerebri and impairment of the venous outflow was likely the result of dilatation of the brain ves-

sels. Thus, MTX therapy is aggressive to the children’s brain and pro-

duces substantial changes in the vascular system adversely influencing BEAB.

Case 1. A 52-year-old man presented in March 2003 with a Ph+ ALL. Induction therapy included high-dose cytarabine and six prophylactic it injections with methotrexate (MTX). He underwent allo-SCT from an unrelated donor after Cy/TBI conditioning. Only four months post-transplant he relapsed in bone marrow (BM) but attained by gradual hearing impairment, without signs of leukemia in periph-

eral blood (PB). In one patient, impaired hearing was the only clinical symptom, whereas the second patient presented a combined hearing and vision loss. Case 1. A 52-year-old man presented in March 2003 with a Ph+ ALL. Induction therapy included high-dose cytarabine and six prophylactic it injections with methotrexate (MTX). He underwent allo-SCT from an unrelated donor after Cy/TBI conditioning. Only four months post-transplant he relapsed in bone marrow (BM) but attained by gradual hearing impairment, without signs of leukemia in peripheral blood (PB). In one patient, impaired hearing was the only clinical symptom, whereas the second patient presented a combined hearing and vision loss. Case 2. A 16-year-old girl was diagnosed with ALL in February 2000. Induc-
	tion treatment included six IT injections, maintenance treatment was ter-

minated after two years. In January 2005, with a 4 week history of pro-

gressive loss of hearing and finally vision, she was referred to hospital. PB counts and differentials were normal. Blasts with ALL immunophe-

notype were found in CSE, confirming a CNS relapse. Pure tone audiom-

ey showed bilateral deafness and audiological tests revealed an audi-

tory neuropathy with normal cochlear function. Despite normal PB
counts, a BM aspiration demonstrated an ALL relapse. Systemic and its chemotherapy was administered, however without any improvement of hearing or vision loss. The patient died of septicemia three weeks after admission. Discussion. The first case illustrates that bilateral hearing impairment may represent the sole symptom of CNS relapse of ALL. The ophthalmic significance of the patient’s hearing problems was overlooked for two weeks after initial biopsy diagnosis was delayed. Interestingly, this patient responded well to it therapy, with improved hearing, and BM disease did not follow CNS relapse. Apparently, inattention, albeit not protecting the patient against CNS relapse, did prevent fulminate hematological relapse. Also in the second case, there was a substantial doctor’s delay due to patient’s initially seemingly harmless symptoms in combination with normal blood counts. In both cases hearing impairment was caused by neuropathy in the auditory nerve. We conclude that hearing impairment in an ALL patient, even if slow, bilateral and isolated, should strongly raise the suspicion of CNS disease.

1373
HEMATOGONES (B-CELL PRECURSORS) AFTER CHEMOTHERAPY IN PATIENTS WITH ACUTE LEUKEMIA BY 3-COLOR FLOW CYTOMETRY
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Backgrounds. Hematogones, B-cell precursors are present in small numbers in the bone marrow and peripheral blood. The immature mononuclear cells including hematogones in the bone marrow aspirates should be differentiated from leukemic blasts in the post-chemotherapeutic bone marrow aspirates with acute leukemia. Annexin V positive and CD34(+) hematogones was evaluated to differentiate them from residual leukemic blasts in the post-chemotherapeutic bone marrow aspirates of patients with acute leukemia. Methods. The bone marrow aspirates (10 hypocellular, 11 complete remission states, 2 persistence states of acute leukemia, 2 post-BMT states) from 25 cases of acute leukemia (7 AML & 18 ALL) were included to measure hematogones by 3-color flow cytometry (CD19-FITC/CD10-PE/CD34-PerCP). Hematogones were defined as the mononuclear cells with coexpression of CD19 and CD10. We analyzed the patterns of hematogones and the correlation of the proportions of total hematogones & more immature CD34(-) hematogones with patients’ ages & the hematologic diagnosis of the bone marrow studies. Results. The groups of patients with less than 1% (N=11, group I), 1-5% (N=7, group II), & equal or more than 5% (N=7, group III) of hematogones in the bone marrow aspirates show 14.5 years, 10.1 years & 8.2 years of the mean ages each, and 6.8%, 43.2% & 48.4% of the mean proportions of CD34(+) hematogones each. We could not find any differences of hematogone patterns between AML and ALL, but according to the post-chemotherapeutic bone marrow states the different findings were noted. In hypocellular marrows and in complete remission states, there were 74.0% & 22.1% of the immature or mature lymphocytes, 26.4% & 25.9% of hematogones among nucleated cells and 12.3% & 55.9% of hematogones among B-cells each. Conclusions. By 3-color flow cytometry (CD19/CD10/CD34) hematogones could be differentiated from residual leukemic cells in the post-chemotherapeutic bone marrow aspirates of patients with acute leukemia. We found that the hematogones, especially more immature hematogones increase more in the younger patients and that the proportions of hematogones are lower in hypocellular marrows inspite of higher proportions of lymphocytes than in complete remission states.

1374
LATE EFFECTS OF CHILDHOOD ALL TREATMENT ON GONADAL FUNCTION IN MALE SURVIVORS
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Current treatment protocols in ALL aim survival with minimal effect on fertility. However, gonadal function is at least finally affected irrespectively of treatment age in males with ALL. The aim of our study was to evaluate gonadal function in our male survivors of childhood ALL in the context of pubertal stages. Subjects consisted of 55 males (9-19 years old) diagnosed between 1975 and 2002 in our department, after a follow up of 10.07±9.00 years. Twenty-nine healthy males of similar chronological age (CA) were taken as controls. Mean CA of the study (Group I=G I) and control (Group II=G II) groups were 15.65±4.79 and 15.42±5.37 years respectively (p=0.34). Forty six patients who had received RT (43; CRT, 2; CSKT) will be indicated as Group IA (G IA). Serum FSH, LH, estradiol (E2), total (T) and free testosterone (FT), inhibin-B, serum hormone binding globulin (SHBG), bilateral testicular ultrasound and semen analysis (in patients > 16 years old, N=24; controls N=9 were evaluated). Pubertal categories (PC) were classified as prepuberty (Tanner stage I=1), early (Tanner stage II-II=2) and late puberty (Tanner stage IV-V=3). Semen analysis was evaluated in the context of categorized sperm counts (azoo/oigoazo- normoazospermia) between GI and GII. Results. Inhibin-B was significantly lower in GIA than in GII in PC 1. Estradiol and FT were significantly higher and SHBG significantly lower in GIA than in GII in PC 3. In conclusion, despite normal inhibin-B levels in early puberty and late puberty, low inhibin-B levels were found in prepuberty. Our findings suggest this might be due to CRT. High E2 and FT levels and appropriately low SHBG levels in late puberty indicate hormonal levels are not yet compromised in these individuals as also reflected by normal FSH, LH, and T levels. Testicular volumes were not reduced in prepuberty, early puberty and late puberty. Sperm counts were not significantly affected.

1375
LYMPHOMAS IN AIDS
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For the last 3 yrs we have followed 51 lymphoma -AIDS pts. 96% were drug users and 80% in association with IV. Non Hodgkin’s lymphomas were diagnosed in 37 pts. Male- 23, female- 14, median age 30. CD4 counts were from 20 to 500 (median 300) cells /mm. Viral load was from 10000 to 500000 copies /mcl. Histological diagnosis by biopsy and postmortem was received in all pts and immunohistochemistry was performed in half of them. Diagnostic laparotomy for lymph node biopsy was done in 6 cases, thoracotomy in 1, orchectomy in 1, splenectomy in 6 pts. The most often diagnosis to be differentiated from in our cases was TBC, established in 40% of lymphadenopathy-AIDS pts Diffuse B- large cell lymphomas happened in 11, Burkitt lymphoma in 6, MALTomas in 4, follicular lymphoma 1, Plasmablastic lymphoma 1, Castleman’s disease 1, T-cell lymphoma 1, primary CNS Lymphoma in one pts. 16 pts had not received treatment and died soon after admission. 21 pts received CHOP (4 with daunoxome), blocks A-B-C of BM- NHL- 95 with CNS prophylactics and Mabthera, ESHAP. Complete remissions were reached in 6 pts, died from lymphoma progression 10 pts, 5 pts are on therapy with good response. Hodgkin’s lymphoma was established in 14 pts. Male- 11, female- 3, median age 30. CD4 counts were from 400 to 1500 cells /mm, VL from 1000 to 100000 copies /mcl. Mixed cellular variant was established in most cases 8 pts had not received polychemotherapy because of late admittance and poorest performance status. Chemotherapy. 11 pts achieved complete remission: on COPP, BOPP, COPP, ABCOPP, 3 pts with CHOP, 1 T-cell lymphoma 1, primary CNS Lymphoma in one pts. 16 pts had not received treatment and died soon after admission. 21 pts received CHOP (4 with daunoxome), blocks A-B-C of BM- NHL- 95 with CNS prophylactics and Mabthera, ESHAP. Complete remissions were reached in 6 pts, died from lymphoma progression 10 pts, 5 pts are on therapy with good response. Hodgkin’s lymphoma was established in 14 pts. Male- 11, female- 3, median age 30. CD4 counts were from 400 to 1500 cells /mm, VL from 1000 to 100000 copies /mcl. Extragonadal germ cell tumors were seen in 3 pts, one female. In one pt 1 yr remission was achieved on BEP therapy. HAART. Conclusion. HIV/AIDS pts with malignant lymphomas may receive diagnostic and treatment approaches which in results may be compared with general population. They must have opportunity to enter general hematological service all the country.

1376
RAPID INFUSION OF RITUXIMAB CAN BE GIVEN SAFELY AND HAS A SIGNIFICANT IMPACT ON CAPACITY
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Administration of Rituximab can be associated with infusion related toxicity. The risk is greatest with the first infusion and lower for subsequent infusions. To minimise the risk of reaction stict infusion guidelines to chemotherapy because of late admittance and poorest performance status. Chemotherapy. 11 pts achieved complete remission: on COPP, BOPP, COPP, ABCOPP, 3 pts with CHOP, 1 T-cell lymphoma 1, primary CNS Lymphoma in one pts. 16 pts had not received treatment and died soon after admission. 21 pts received CHOP (4 with daunoxome), blocks A-B-C of BM- NHL- 95 with CNS prophylactics and Mabthera, ESHAP. Complete remissions were reached in 6 pts, died from lymphoma progression 10 pts, 5 pts are on therapy with good response. Hodgkin’s lymphoma was established in 14 pts. Male- 11, female- 3, median age 30. CD4 counts were from 400 to 1500 cells /mm, VL from 1000 to 100000 copies /mcl. Extragonadal germ cell tumors were seen in 3 pts, one female. In one pt 1 yr remission was achieved on BEP therapy. HAART. Conclusion. HIV/AIDS pts with malignant lymphomas may receive diagnostic and treatment approaches which in results may be compared with general population. They must have opportunity to enter general hematological service all the country.
for infusion related reactions. All patients received pre medication of Paracetamol 1 gm orally, Chlorpromazine 10mg IV and Hydrocortisone 100 mg IV, 30 minutes prior to commencing Rituximab. Results. All 16 patients received the 1st infusion at the standard rate with no adverse effects. To date 75 subsequent infusions have each been administered over 90 minutes. This schedule has been extremely well tolerated with no grade 1-4 reactions noted. Conclusion. The shorter infusion schedule for Rituximab is well tolerated and safe and has had a significant impact on capacity problems in the day therapy unit.

1377
A RETROSPECTIVE ANALYSIS OF 57 CASES OF MANTLE-CELL LYMPHOMA ADMITTED IN THE CLINIC OF HAEMATOLOGY- FUNDENI INSTITUTE BETWEEN 1994-2004
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Background. Mantle-cell lymphoma (MCL) represents a problem for the haematologist due both to the difficulty in establishing the diagnosis and to the lack of response to standard lymphomas protocols therapy. Aims. The aim of this study was to analyze the clinical aspects, the identification of major prognosis factors as well as the therapeutic results in 57 cases of MCL admitted in the Clinic of Haematology-Fundeni between 1994-2004. Methods. The diagnosis was based on the histological examination (WHO criteria) and/or immunophenotyping tests (CD5+, CD23-). Results. Their median age was 61 years, M/F rates 1.2; 81% of patients presented in an advanced stage of disease (stage III-IV Ann Arbor) with generalized adenopathies (90% of cases, 72% predominantly axillary) but with a good performance status (75%, with ≤ 2 ECOG). Bulky disease was detected in 26% and extranodal determinations ≥ 2 in 84% of cases. Lymphocytosis ≥ 4.000/μL in 81% and ≥ 10.000/μL in 51% of cases, anaemia (Hb < 12 g/dL) in 50% and bone-marrow involvement in 75% of cases. Other extranodal localizations were recorded in gastrointestinal tract (15%), liver (26%), pleura (17%), Waldeyer ring (7%), skin (7%), orbital space (1%) and Nervous Central System (1%). LDH had increased values over normal limits in 64%. M-component in blood in 14%. 68% had an IPI score > 2 and 32% an IPI score ≤ 2. The initial therapy started with Chlorambucil+Prednison (16 cases); CVF/COP (21 cases) and CHOP or CHOP-like (21 cases). Fludarabine+ Cyclophosphamide were introduced only in relapsing or in refractory diseases in 9 cases. Rituximab was administered in 3 cases (one administration per week) and R-CHOP-like was given in 1 case. Splenectomy was carried out in 3 cases. α interferon was applied in 4 cases (3 administration x 3 MU each per week) as a maintenance therapy. In 23 cases (40%) complete and partial remissions were obtained. The median survival time for the whole lot was 20 months (3 - 103 months). The univariant analysis revealed that good performance status (ECOG 2), limited clinical stages (I, II) no bulky disease, hemoglobin level >12 g/dL, normal values of LDH and IPI ≤ 2 were major predictive factors correlated with long survival. Conclusions. Mantle-cell lymphoma remains a problem of diagnosis and therapy; the evolution is ineluctable fatal as the disease is largely disseminated at presentation, is generally resistant to standard therapy with a median survival of 56 years (range 27-88 years). At diagnosis 93% of patients had good performance status (ECOG-2) and 5 (22%) had B-symptoms. 7 patients (30%) had anaemia and 12 patients (52%) had elevated levels of LDH. 16 patients (70%) with stage I and II, 7 patients (30%) with stage II and IV were admitted. 12 (92%) of gastric localization of MALT lymphoma and 11 (48%) non-gastric localization (7 salivary gland, 2 lung, 1 thyroid, 1 colon) were observed. None of the patient had bone marrow involvement. Results. All the patients are alive with a median 28 months (range 2-150 months) of follow-up. 4 patients (17%) received doxorubicin based systemic chemotherapy, 4 patients (17%) radiotherapy and 2 patients (8%) antibiotic therapy for HP eradication only. 10 patients (43%) were treated with complete surgical excision (3 stomach, 5 salivary gland, 1 lung, 1 thyroid), 4 of them combined with chemotherapy (3 stomach, 1 salivary gland), 6 of them combined with radiotherapy (4 salivary glands, 1 thyroid, 1 lung), 2 patients with lymphoma of stomach localization were treated with antibiotics followed by localized radiotherapy. One patient has been followed-up alive without treatment because he didn’t accept treatment. All the treated patients achieved complete remission (95%) except one who achieved partial remission. Summary/Conclusions. Because of the indolent course the prognosis of MALT lymphoma was good regardless of the treatment modalities. The role of the final management of the disease has not been clearly defined. The treatment choice should be patient-tailored, taking into account the site, stage, age and other clinical characteristic of patient.

1379
EVALUATION OF PERIPHERAL BLOOD AND BONE MARROW INVOLVEMENT IN MANTLE CELL LYMPHOMA (MCL) - IMMUNOPHENOTYPIC AND MORPHOLOGICAL FINDINGS
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MCL is a B-cell malignancy with distinct molecular genetics and pathological features. It has been reported that advanced Ann-Arbor stage IV and especially leukemic phase of the disease are associated with poorer prognosis. This study aimed to evaluate immunophenotypic and morphological lymphoma cells in bone marrow (BM) and/or peripheral blood (PB) in MCL patients (pt) with clinical stage (CS) IV and V (leukemic phase) in comparing with morphological and clinical features. Forty pts were studied (med. age 62, range 37-82 y, M/F=1:56:1) from 1996-2005. Overall survival (OS) for all pts was 21.5±18.7 (4-52 months). 17 (42.5%) of the IV CS pts had the leukemic phase of the disease. Presenting features included splenomegaly (75%), hepatomegaly (19%), lymphadenopathy (50%), gastro-intestinal and sinusues involvement (6%), respectively. Mean hemoglobin (Hb) was 11±24 g/l, platelets 131±9 ×10⁹/l, and leukocytes 51 ± 66 ×10⁹/l. The pattern of marrow biopsy involvement was diffuse (47.5% pts), interstitial (30% pts), nodular (15% pts) and paratrabeular (7.5% pts). Morphological blastoid variant of MCL was found in 7 pts (17.5%), whereas the rest of pts had small cells and standard MCL cells morphology. Immunophenotyping and multiparameter flow cytometry were done on the PB (67.5%) or BM (12.5%) samples. Surface markers were identified using monoclonal antibodies against CD1, CD19, CD20, CD22, CD10, FMC7, CD5, CD23, CD8, CD79b, CD2, CD3, kappa, and lambda antigens (Ag). Immunological markers showed a typical expression pattern in all patients: CD19+, CD20+, CD5+, CD3-, CD4+, CD8+, CD45RO+, CD25+, CCR7-, CD38-, CD39-. The results of the current study demonstrated that among the whole group of pts (Hi square test, p<0.05). The results of the current study demonstrated that among the whole group of pts with CD5+, CD38- orders in response to either infection such as Helicobacter pylori (HP) gastritis in the stomach, or autoimmune process like Hashimoto’s thyroiditis, Sjogren’s syndrome. We conducted this study to demonstrate our experience in patients with MALT lymphomas and compare our results with the literature. Patients and Methods. We retrospectively studied 23 patients with MALT lymphomas of different localizations, treated with different modalities over a period time ranging between 1992 and 2005. The female/male rate was 15/8 and the median age at diagnosis of 56 years (range 27-88 years). At diagnosis 93% of patients had good performance status (ECOG-2) and 5 (22%) had B-symptoms. 7 patients (30%) had anaemia and 12 patients (52%) had elevated levels of LDH. 16 patients (70%) with stage I and II, 7 patients (30%) with stage II and IV were admitted. 12 (92%) of gastric localization of MALT lymphoma and 11 (48%) non-gastric localization (7 salivary gland, 2 lung, 1 thyroid, 1 colon) were observed. None of the patient had bone marrow involvement. Results. All the patients are alive with a median 28 months (range 2-150 months) of follow-up. 4 patients (17%) received doxorubicin based systemic chemotherapy, 4 patients (17%) radiotherapy and 2 patients (8%) antibiotic therapy for HP eradication only. 10 patients (43%) were treated with complete surgical excision (3 stomach, 5 salivary gland, 1 lung, 1 thyroid), 4 of them combined with chemotherapy (3 stomach, 1 salivary gland), 6 of them combined with radiotherapy (4 salivary glands, 1 thyroid, 1 lung), 2 patients with lymphoma of stomach localization were treated with antibiotics followed by localized radiotherapy. One patient has been followed-up alive without treatment because he didn’t accept treatment. All the treated patients achieved complete remission (95%) except one who achieved partial remission. Summary/Conclusions. Because of the indolent course the prognosis of MALT lymphoma was good regardless of the treatment modalities. The role of the final management of the disease has not been clearly defined. The treatment choice should be patient-tailored, taking into account the site, stage, age and other clinical characteristic of patient.
CD34- phenotype, pts with blastoid variant had significantly higher proportion of cells with CD25 expression, despite of pts with standard cytological morphology. Differences in immunophenotype between pts with blastoid variant and small cells or typical MCL cells, deserve prospective analyses in large cohort of pts, and give some insights about their biological features.

1380

IS GASTRECTOMY NECESSARY FOR NON-HODGKIN WITH GASTRIC INVASION?

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Aim: CHOP or R-CHOP has been established as a standard first-line chemotherapy for non-Hodgkin lymphoma (NHL). On the other hand, gastrectomy with postoperative chemotherapy for NHL of stomach has been considered as a standard therapy same as for gastric cancer. Recently, there are some reports that chemotherapy and radiation therapy (RT) for NHL of stomach has same or more survival rate compared with gastrectomy. In this study, we investigated the utility of chemotherapy and the necessity of gastrectomy for the NHL patients with gastric invasion. From 1994 January to 2005 October, 91 NHL patients were admitted to our hospital. With endoscopic examination, they were grouped in NHL groups with gastric invasion (GI) (n=19) and without gastric invasion (NGI) (n=71). The average of GI age was 65±10, and NGI was 62±13. Gender (M:F) was GI (7/6) and NGI (44/34). PS(0,1>2) was GI(9) and NGI(46/32). According to Ann Arbor classification, Grade I and II were defied as a mild group, Grade III and IV were defied a severe group. Seventy (mild to severe) were GI (21) and NGI (26/52). Pathological diagnosis of DLBCL was 10 cases (77%) in GI, 54 cases (69%). Average of blood Hb was 11.7±1.1 g/dl in GI and 11.8±1.8 in NGI. Average of albumin was 3.6±0.6 in GI and 3.5±0.6 in NGI. CHOP and R-CHOP were given in 6 cases and 7 cases of GI, 69 cases and 9 cases of NGI. There were no differences between GI and NGI in background of patients. There was significantly no difference cumulative survival rate in between GI and NGI. Also, in group GI, there was no difference cumulative survival rate in between mild group and severe group. It was suggested that chemotherapy of CHOP or R-CHOP is effective for NHL patients, whether there was gastric invasion or not. Gastrectomy might not be necessary for NHL patients except emergency and special cases.

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CEOP RITUXIMAB: AN EFFECTIVE AND SAFE REGIME FOR ELDERLY PATIENTS > 75 YEARS OLD WITH DIFFUSE LARGE B-CELL LYMPHOMA


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Backgrounds. About one quarter of diffuse large B-cell lymphomas (DLBCL) affects individuals of 75 years of age or older. Nevertheless, data on optimal treatment of these patients is scarce, mainly because very elderly patients are usually excluded from clinical trials due to poor performance status and co-existing medical conditions. According to previous studies, replacement of adriamycin with epirubicin in CHOP regimen has proven equally effective in the treatment of aggressive non-Hodgkin lymphomas (NHL), yielding at the same time lower rates of cardiac and hematological toxicity. Rituximab, on the other hand, is well-known for its effectiveness and good tolerance in the treatment of elderly patients with aggressive NHL. Aim: To study retrospectively the efficacy and safety of CEOP regimen ± rituximab in very elderly patients with DLBCL. Methods. Between 1999 and 2005, 45 patients, 20 (44.4%) males and 25 (55.6%) females with median age of 78 (75-85) years were diagnosed with DLBCL in our department. Twenty-one (46.7%) of them had DLBCL of nodal origin and 24 (53.3%) of primary extranodal origin. Twenty-seven (60%) patients presented with early stage (I-II, no X) disease and 35 (77.3%) with IPI 1-2. Nine (20%) patients had B symptoms, 3 (6.7%) bulky disease, 2 (4.4%) bone marrow infiltration and 12 (26.7%) extranodal involvement other than primary. All patients received cyclophosphamide 750 mg/m² IV, epirubicin 62.5 mg/m² IV and vincristine 1.4 mg/m² IV on day 1 and prednisone 75 mg IV on days 1-5. Doses were reduced mild to severe by age > 80 years. Medical history of heart disease, by 25% in 15 (33.3%) and by 50% in 8 (6.7%) patients. Rituximab was additionally administered on day 1 of each chemotherapy cycle at a dose of 375 mg/m² IV, in 23 (51.1%) patients. Fifteen (53.3%) patients also underwent radiation therapy. All patients were under close hematicological and cardiac monitoring throughout treatment. Disease-free survival (DFS), overall survival (OS) and failure-free survival (FFS) were estimated according to the Kaplan-Meier method. Results. Median follow-up time was 30 (1-105) months and median number of chemotherapy cycles administered was 6 (1-8). On an intention-to-treat basis complete response was observed in 33 (73.3%) patients, partial response in 5 (11.1%), stable disease in 2 (4.4%) and progressive disease in 1 (2.2%), whereas 6 (13.3%) patients were lost before they could be evaluated. Sixteen (35.6%) patients are dead and 27 (60%) are alive, 17 (63%) of which, in remission. Actuarial DFS, OS and FFS rates at 3-years were 75.8%, 67.3% and 53.5% respectively. OS and FFS rates at 5-years were significantly (p<0.003) higher in responders (76.7% and 60.3% respectively) than non-responders (19.3% and 22.9% respectively). No treatment-related deaths were noted, while hematological and cardiac toxicity remained acceptable. Conclusion: CEOP ± rituximab is a feasible, safe and effective treatment for very elderly patients with DLBCL. The high response and survival rates in our study justify the right of these patients to a potentially curative treatment.

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EFFICACY OF EPOCH AND RITUXIMAB-EPOCH AS SALVAGE THERAPY FOR RELAPSED OR REFRACTORY B-CELL LYMPHOMA: PRELIMINARY RESULTS OF AN OPENED, NON-RANDOMIZED STUDY IN A SINGLE CENTER

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Backgrounds. Relapsed or refractory B-cell Non-Hodgkin lymphoma (NHL) have a poor prognosis. EPOCH chemotherapy and rituximab-EPOCH (R-EPOCH) regimen have been used in recent years as salvage therapies in such cases. Aims: To preliminarily evaluate efficacy of EPOCH and R-EPOCH regimens in terms of response rate, survival and toxicities. Possible predictive factors for response were also assessed. Methods. Thirty-six patients with relapsed or refractory B-cell NHL were enrolled in Songkananagnd Hospital during January 2003 and January 2006. All of patients received conventional CHOP chemotherapy without rituximab as a first-line treatment. In an opened, non-randomized way, 25 patients received EPOCH (doxorubicin 10 mg/m², etoposide 50 mg/m², vincristine 0.5 mg as a continuous IV infusion on days1-4, cyclophosphamide 750 mg/m²/IV on day 5 and prednisone 60 mg/m² orally on days 1-5) and 11 patients received R-EPOCH (rituximab 375 mg/m² intravenous for 2 hr before EPOCH regimen). Results. The patient characteristics were not statistically different between EPOCH group and R-EPOCH group, in terms of age, gender, histopathology, stage of disease, IPI, B-symptoms, performance status, bulky mass, elevated LDH, prior response to the first-line chemotherapy, and duration from diagnosis to salvage treatment. Of 23 evaluable patients with EPOCH treatment and of 11 patients with R-EPOCH, objective responses were obtained in 52% (35% CR, 17% PR) and 75% (64% CR, 9% PR), respectively (p<0.30). There were no significant predictive factors of response as a function of histopathology, stage of disease, IPI, B-symptoms, performance status, bulky mass, elevated LDH, prior chemotherapy, history of chemotherapy-related reactions occurred in 2 patients (18%). Febrile neutropenia developed in 12 of 216 cycles (6%). Cardiotoxicity was around 8%. Because of short duration of follow-up (median, 7.8 mo for EPOCH and 10.6 months for R-EPOCH), EFS and OS could not be appropriately analysed at this report. Conclusion: EPOCH and R-EPOCH regimens were effective and well tolerated in patients with B-cell NHL who were relapsed or resistant to the conventional chemotherapy. R-EPOCH seemed to give higher response rate that EPOCH but it is not statistically significant. Because of this preliminary result, more number of patients and longer duration of follow-up are needed.

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IN VIVO AND IN VITRO PURGING WITH RITUXIMAB PLUS CHEMOTHERAPY, CD34+ CD133+ CELL SELECTION AND HIGH DOSE CHEMOTHERAPY AS CONSOLIDATION TREATMENT IN ADVANCED MANTLE-CELL LYMPHOMA


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Backgrounds. Mantle-cell lymphoma (MCL) remains an incurable lymphoproliferative disease with standard chemotherapy resistant (CT). The chimeric monoclonal antibody anti-CD20 Rituximab has demonstrated to improve the CR rate representing an interesting in vivo purging modality, however most pts treated with standard CT relapse within 2 years and the role of consolidation therapy is crucial. In vitro purging might contribute in obtaining a molecular (bc1-1) CR which could be predictive

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for improved outcome. Aims. Here we considered the efficacy of an intensified in vitro purging protocol and as maintenance therapy, could represent a new avenue to improve the engraftment especially for platelet recovery. The possibility to achieve a CR and conversely purging consolidation is a critical step in the treatment of MALT gastric NHL. Although the number of patients enrolled is still low, we report results of a pilot study of Zevalin delivered at standard dosage to receive it. RIT with Zevalin has demonstrated activity in follicular (FL) but also in MCL. Our data seem suggestive of a more active role of Zevalin in patients with MALT gastric NHL resistant or refractory to conventional systemic treatment. Its mechanism of action mimics conventional RT already known as valid option in MALT GASTRIC NON HODGKINS LYMPHOMA

To verify efficacy and confirm safety profiles of standard RIT regimens and as maintenance therapy, could represent a new avenue to improve the engraftment especially for platelet recovery. The possibility to achieve a CR and conversely purging consolidation based on Rituximab delivered at standard dosage for improved outcome. Aims. Here we considered the efficacy of an intensified in vitro purging protocol and as maintenance therapy, could represent a new avenue to improve the engraftment especially for platelet recovery. The possibility to achieve a CR and conversely purging consolidation is a critical step in the treatment of MALT gastric NHL. Although the number of patients enrolled is still low, we report results of a pilot study of Zevalin delivered at standard dosage to receive it. RIT with Zevalin has demonstrated activity in follicular (FL) but also in MCL. Our data seem suggestive of a more active role of Zevalin in patients with MALT gastric NHL resistant or refractory to conventional systemic treatment. Its mechanism of action mimics conventional RT already known as valid option in MALT GASTRIC NON HODGKINS LYMPHOMA

Efficacy of 90Y ibritumomab tiuxetan (Zevalin) in Refractory or Relapsed MALT Gastric Non Hodgkins Lymphoma


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Backgrounds. Radio-immunotherapy (RIT) has been developed to vehiculate radionucleides in a selective manner to tumor cells targeted by monoclonal antibodies. The rationale for the use of this approach is the opportunity to deliver radiotherapy (RT) in the tumor bulk avoiding the exposure of other tissues. Toxic effects are well known and limited prevalently to the hematologic counterpart, but transient and reversible. Though, this modality treatment could be offered also to those patients who had previously received RT or who are not suitable to receive conventional RT and who are considered as candidates for a second line therapy. Zevalin has demonstrated activity in follicular lymphoma and diffuse large B-cell (DLBCL) NHL, but data are still lacking in MALT NHL. We report results of a pilot study of Zevalin delivered at standard activity (0.4 mCi/kg) in gastric NHL pts relapsing/resistant after standard therapies. Aims. To verify efficacy and confirm safety profiles of standard dose Zevalin in gastric NHL resistant or refractory to conventional systemic therapy. Results. From May 2004 to January 2006 7 patients were enrolled. They all had gastric resistant/refractory CD-20 positive B-cell NHL: 2 were DLBCL and 5 were MZL. Median age was 57 yrs (range 36-64), 3 female and 5 male. At time of treatment median 5 out of 7 patients had stage II/III (MALT MZL), while 2 out of 7 pts had stage II/IV (DLBCL) of disease. Median number of previous therapies received was 2 (1-4); all of them had received prior RT, 3 prior Rituximab, no one had received prior RT. Toxicities were mild (G2 NCl), reversible after 6 weeks from therapy and primarily hematological. Six out of 7 patients are now evaluable for responses: 4 CR (all MALT MZL), 2 PD (both DLBCL NHL). Conclusions. Based on such preliminary results, Zevalin seems to be very active principally in pts with MALT gastric NHL resistant or refractory to conventional systemic therapy. Its mechanism of action mimics conventional RT already known as valid option in MALT gastric NHL. Although the number of patients enrolled is still low, we are continuing our experience in order to confirm such results. However, with our single institution of Zevalin delivered at standard dosage could be considered a possible alternative option in the treatment of such indolent disease. Updated data will be presented and discussed.

Combinant Urate Oxidase (Rasipuricase) for the Prevention and Treatment of Hyperuricemia During Chemotherapy for Hematological Malignancies

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Tumor lysis syndrome (TLS) is a serious complication of the induction therapy of lymphomas and leukemias. The standard approach for the prevention and management of hyperuricemia is hydration, oral allopurinol and alkalization. Urate oxidase is an enzyme that catalyses the conversion of uric acid (UA) to allantoin which is 5-10 times more soluble than UA and therefore is excreted by the kidneys more easily. It is found in most mammals but not in humans. Rasipuracase, a recombinant form of urate oxidase catalyses the conversion of uric acid to allantoin and therefore controls hyperuricemia faster and more efficiently than allopurinol. To evaluate retrospectively the safety and efficacy of rasipuracase in patients with hematological malignancies who were treated with chemotherapy and had increased risk to develop tumor lysis syndrome. We studied 21 patients (16 men and 5 women) with median age 63 years (range 26-76). They had Burkitt lymphoma (n=5), lymphoblastic lymphoma (n=2), mantle cell lymphoma (n=1), diffuse large B-cell lymphoma (n=5), Hogdkin lymphoma (n=2), chronic lymphocytic leukaemia (n=4), acute leukaemia (n=4) and received rasipuracase during the first therapy for their disease. Before treatment 76% of the patients had increased lactate dehydrogenase (LDH), and 52% increased uric acid. Rasipuracase was administered intravenously at a dose of 0.20 mg/kg/day for 2 to 7 days, starting from the first day of any chemotherapy. Uric acid, electrolytes, urea and creatinine were measured every day during therapy. All patients responded to therapy with rasipuracase. The mean UA level in 11 hyperuricemic patients decreased from 10.7 mg/dl to 0.6 mg/dl after 24 hours. Prophylactic administration of rasipuracase to 10 patients with normal UA level reduced the mean UA level from 5 mg/dl to 0.4 mg/dl after 24 hours (for all reductions in UA levels p<0.001). We did not observe any increase in the levels of creatinine or any electrolyte disturbances. One patient presented fever as an adverse event which subsided after the interruption of the rasipuracase. The results of this study confirm the efficacy and safety of rasipuracase for the prevention and treatment of hyperuricemia induced by chemotherapy in patients with hematological malignancies.

Body Composition Changes During Chemotherapy of NHL

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Weight loss, anorexia, and pain are common symptoms in patients with malignancies. Metabolic changes induced in the host lead to loss of body mass, breakdown of muscle and adipose tissue, leading to increased risk of infections, decreased quality of life and poor response to chemotherapy. The clinical course of a cohort of patients diagnosed non-Hodgkin lymphoma (NHL) and treated with CHOP was evaluated to determine changes in body composition during chemotherapy. The study sample included 30 patients with immunohistochemically confirmed NHL that were treated with CHOP chemotherapy regimen. Before first, third and sixth cycle body weight was measured along with skinfold thickness on four places (by Harpenden caliper) and bioelectric impedance analysis was done (four electrode technique proposed by Hofer et al.) (BIA). Body weight during chemotherapy shows constant but statistically insignificant increase in all patients (mean weight before therapy 69.5±6.2kg and after completion of therapy 71.6±1.1kg). Body fat percentage determined by Durning and Rahaman method shows statistically significant correlation to fat mass percentage measured with BIA that enabled usage of other results computed by BIA. Body fat percentage was (mean measures) 24.85 before therapy and 27.72 before sixth cycle (statistically insignificant increase) in patients with response (ORR 86.7%), and 39.2 to 30.9 in patients with no response to therapy (13.3%). Percentage of lean body mass shows opposite results - decrease during chemotherapy (also statistically insignificant). Patients in this study show unfavorable changes in body composition (increase of fat mass and decrease in lean body mass). Since these changes could be important in well known trial dose-toxicity-response relations...
1387 PLATINUM-BASED PROTOCOLS IN THE TREATMENT OF RELAPSED OR REFRACTORY HODGKIN DISEASE PREVIOUS TO HEMATOPOIETIC PROGENITORS TRANSPANTATION


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The platinum-based protocols (ESHAP or EDHAP) have been widely used in relapsed or refractory cases of non-Hodgkin lymphoma (NHL) and even as a conditioning regimen prior to transplantation as well. In contrast to these data, their use in Hodgkin disease is scanty. We present our experience in 5 patients with relapsed or refractory Hodgkin disease treated with these protocols all of them prior to transplantation. Five patients (4 males, 1 female), mean age 41.2 years (25-55) are presented. Histologic subtypes included 3 lymphocyte predominance and 2 nodular sclerosis. Three cases were relapsed and 2 resistant to previous therapies. Four patients were treated with ESHAP cycles and only one with EDHAP. No modifications on the conventional doses or schedule of these protocols were made. In two cases with lymphocyte predominance histology Rituiximab (375 mg/m2, day 1) was added to the conventional protocol. A total of 18 cycles were administered, mean number of cycles was 6,6 (2-5). Four patients received complete doses and in one case cycles were reduced 50% due to previous hypertension. No delayed administration of cycles was observed. Only one patient required G-CSF due to neutropenia. Toxicity included (for 18 cycles): 6 events of grade I-II anemia; 1 case of grade I-II and 3 cases of grade III-IV neutropenia; 4 cases of grade III-IV thrombocytopenia. All neutropenias and thrombocytopenias were observed in the same patient, who presented two febrile episodes as well. Only one patient presented grade I renal toxicity after 3 cycles and other presented hypomagnesemia after 2 cycles. Response was observed in all cases (5 CR and 2 PR). Three patients have received autologous transplantation (other has been proposed but not performed yet), and the other a non-relievable aleuagenic one from his sister. In those cases of autologous procedures enough number of CD34+ cells could be obtained from peripheral blood (2 cases) or bone marrow (1 case) without significative problems. All but one patient (autograft) remain in CR after transplantation. In our experience, platinum-based protocols are a safe, well tolerated and worthwhile option for the treatment of patients with refractory or relapsed Hodgkin disease. These protocols do not seem to affect the number of CD34+ cells collected in cases of autologous transplantation.

1388 HODGKIN’S DISEASE IN CHILDREN: CLINICAL CHARACTERISTICS, TREATMENT RESULTS AND PROGNOSTIC VALUE OF SERUM COPPER LEVEL IN A SINGLE INSTITUTION IN CENTRAL ANATOLIA

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Backgrounds. Hodgkin’s Disease (HD) is one of the common malignancies in childhood. There is limited information regarding the management of HD in developing countries. Aims. The aim of this study is to analyze clinical characteristics, histology, staging, and treatment results on children presenting with HD and to investigate retrospectively pre-treatment factors such as copper level that might influence survival rates.

Patients and Methods. From March 1988 to May 2004, 42 Turkish children with biopsy-proven HD who were younger than 16 years of age were retrospectively included into the study. The age range of children was 3 months-16 years (mean age 6.2 years). The male to female ratio (29 male and 13 female) was 2.2:1. Treatment Protocols up to 2000, COPP was modified to COPP scheme or CHPP regimen were started for 5 patients with refractory or relapsed Hodgkin disease.


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This is a retrospective study of the paediatric patients cohort with Hodgkin Lymphoma diagnosed and followed up in a single center, during the years 1997-2000. The aim of study is to present epidemiological data, clinical characteristics, clinical and histological staging, response to chemotherapy and define the role of irradiation and the final outcome. Patients and results: During the last 8 years 24 children, 17 boys and 7 girls, aged 4.5 to 15 years old were diagnosed with Hodgkin Lymphoma in the Department of Pediatric Hematology - Oncology of the Agia Sofia Hospital. Eight out of 24 patients (35%) presented with B-symptoms, while 16/24 have been admitted with signs of inflammation of cervical and supraclavicular lymph nodes. The 3 out of 8 patients with B-symptoms did not presend peripheral lymphadenopathy. In all our patients the diagnosis was established with biopsy material. In all patients bone marrow biopsy was performed. Of note, one of them was diagnosed by bone marrow involvement. Twenty out of twenty - four (20/24) and 19/24 underwent Gallium lymph node and Technetium bone Scann, respectively. Regarding pathology type, 17/24 patients had nodular sclerosing type, six (6) mixed cellularity and one (1) lymphocyte predominant histology. Thirteen (13/24) patients had positive Gallium Scann and while three (3/24) were positive for bone involvement by Technetium Scann. Clinical staging: 3 children were staged as stage I (3/3 IA), 10 stage II (3/10 IIB), 6 stage III (4/6 IIIA) and 5 stage IV, 15/24 (19/24) of the patients were treated according to the German protocol GPOH-HD-95, while 5 were treated according to the Danish protocol GPOH-HD-95. Overall 23/24 patients are alive, 1 patient died 24 months after the diagnosis. Conclusion. We report the survival of our patients with Hodgkin Lymphomas after their first or second remission is standing high. More patients are salvage following relapse. The longterm follow-up of children with HD concerning possible complications of therapy remains a serious issue. We believe this study offers useful clinical information about staging, appropriate treating decision and outcome of the disease in the longterm inclusion.

1390 COMBINATION STUDIES OF LENALIDOMIDE WITH CHEMOTHERAPEUTIC AGENTS ON THE PROLIFERATION OF THE CHROMOSOME 5 DELETED BURKITTS LYMMPHOMA NAMALWA CSN.70 CELL LINE

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Backgrounds. Lenalidomide (Revlimid®), has recently been approved for the treatment of a subset of myelodysplastic syndromes (MDS), and is currently being evaluated as treatment for other hematologic and oncology conditions, including multiple myeloma, chronic lymphocytic leukemia as well as solid tumor cancers. Lenalidomide efficacy has been reported in clinical trials of MDS patients with a 5q- cytogenetic abnormality, with or without other cytogenetic abnormalities. Aims. The present study evaluates the combinational effects of
lenalidomide in the lenalidomide-sensitive chromosome 5 deleted Burkitt’s Lymphoma tumor cell line, Namalwa CSN70, with various chemotherapeutic agents used for oncological treatment (cyclophosphamide, doxorubicin, vincristine, methotrexate, cytarabine, ifosfamide, carbustine, prednisone and etoposide). Methods. Cells were incubated in 96-well cell culture plates with compounds for 72 hours and assayed by 3H-thymidine incorporation. IC50s were calculated by nonlinear regression (GraphPad Prism). Results. Namalwa cell proliferation. Specifically, lenalidomide in combination with cytarabine, doxorubicin, or vincristine generated anti-proliferative responses that were equivalent to the inhibition produced by these respective chemotherapeutic agents alone. Lenalidomide in combination with cyclophosphamide or ifosfamide produced anti-proliferative effects similar to lenalidomide alone. These data indicate non-additive effects for the previously mentioned lenalidomide/chemotherapeutic agent combination. However, the lenalidomide/etoposide combination yielded partially additive anti-proliferative effects within the concentration range of 0.05 and 0.5 mM. At higher concentrations, > 0.5 mM, the response became non-additive and comparable to the etoposide treatment alone. Also, the lenalidomide/prednisone combination resulted in partial additive anti-proliferative effects but at concentrations > 0.5 mM and was non-additive at lower concentrations. Antagonistic effects were observed with the lenalidomide/carmustine combination at low concentrations while partially additive anti-proliferative effects were observed at higher concentrations. Antagonistic effects were also observed with the lenalidomide/methotrexate combination. Conclusions. Together, these results suggest a possible beneficial anti-proliferative response for lenalidomide in combination with either etoposide or prednisone against diseases such as del 5q MDS.

Prognostic Significance of Ki67 and Clinical Parameters in Classical Hodgkin’s Lymphoma

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Background. The growth fraction of the malignant cells, International Prognostic Score (IPS), as well as the other clinical and laboratory parameters in classical Hodgkin’s disease, tissue eosinophilia and red blood cell sedimentation rate-ESR are considered to have prognostic relevance in Hodgkin’s lymphoma (HL). Aims. To evaluate proliferation index in Hodgkin and Reed-Sternberg (HRS) cells by Ki67 labeling, to determine the prognostic value of other clinical and laboratory parameters including IPS in cohort of patients with HL. Their significance was evaluated according to response to treatment and survival period. Optimal initial prognostic model was determined according to these findings. Methods. A retrospective study was performed on cohort of 40 pts (20 male/20 female) aged 18-85 yrs and prednisone and etoposide treated. The median follow-up was five years (yrs). All pts were treated according to standard clinical approach, ABVD regimen. The expression of proliferative marker Ki67 was determined by immunohistochemistry in formalin fixed, paraffin embedded tissue biopsy specimens at diagnosis. We analyzed the percentage of HRS cells with Ki67 positive (+) nuclear staining on 10 different high power microscopy fields (x 400). Results. Twenty percent of pts were in stage I and II and 80% were in advanced stages III and IV. The mean age was 55.3±10.3 yrs (75% of pts were <45 yrs). The overall survival rate was 72.5% after 5 yrs of follow-up. Pts with high proliferative fraction (Ki67+ ≤50%) had worse survival (OS 56%) compared to those with low proliferation (Ki67+ >50%) with OS 91% (Log Rank test p<0.01). There was not statistically significant correlation between Ki67+ and the achievement of complete remission (p>0.05). Cox’s multivariate analysis revealed that Ki67+ at threshold of 50% was significant independent prognostic factor (p=0.015). From clinical parameters, ESR had a negative trend concerning remission rate and overall survival. Bulky disease, tissue eosinophilia and ESR>50 had no significant effect on complete remission rate and overall survival. However, there was trend of divergence in Kaplan-Meier’s survival curves after a four years follow-up (log rank p>0.05). Conclusions. The patients with high Ki67+ are at risk of relapse and treatment failure, and are eligible for the initial aggressive therapeutic approach.

INCIDENCE AND CLINICAL SIGNIFICANCE OF AUTOIMMUNE COMPLICATIONS IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA AND NON-HODGKIN’S LYMPHOMA

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Background Autoimmune complications (AC) as autoimmune hemolytic anemia (AIHA) and immune thrombocytopenic purpura (ITP) can be present in evolution of chronic lymphoproliferative disorders (CLPD) or they, sometimes, precede the diagnosis. The outcome of patients with CLPD associated with AIHA or ITP has been reported, in previous studies, to be similar to the outcome of patients without AC. The aim of our study was to evaluate the incidence and clinical significance of AC in patients with B-cell chronic lymphocytic leukemia (CLL) and in patients with non-Hodgkin’s lymphoma (NHL). Methods. In a retrospective analysis we studied 384 patients with CLL (165 pts) or NHL (219 pts) diagnosed and treated in a single institution between 1990-2004. Clinical and hematological data were reviewed for patients with AC, classified of incidence, clinical response, and disease stage, histological type, and treatment. Results. AC were found in 31 of 384 patients (8.07%), in 17 (10.30%) of 165 CLL patients and in 14 (6.39%) of 219 NHL patients. AIHA was present in 18 patients (58.06%), ITP in 7 patients (22.5%), while in 6 patients (19.35%) an Evans syndrome (AIHA associated with ITP) was diagnosed. AC were more frequent in CLL than in NHL (AIHA: 70.58% vs 42.85%; ITP: 29.41% vs 14.28%). In 3 cases (9.67%; 2 CLL and 1 NHL) AIHA was diagnosed before the diagnosis of CLPD. Most of AC appeared in the advanced stages of the diseases (76.47% CLL stage C; 64.28% NHL stages III-IV). The median lymphocyte count at diagnosis, in the 17 patients with CLPD was 48.9×10^9/L (7.8 - 188.1). Of the 14 patients with NHL, AC were diagnosed in 3 cases (21.42%) with small lymphocytic lymphoma, in 2 cases (14.28%) with follicular lymphoma, and in 9 cases (64.28%) with diffuse large-B cell lymphoma. The successful treatment of AC included corticosteroids (96.77%) and/or chemotherapy (19.35%). The outcome of patients with AC was not different from the outcome of patients without AC. Nine (52.94%) of the 17 CLL patients, and 8 (57.14%) of the 14 NHL patients reached a complete response after adequate chemotherapy. The median survival rates were 4.8 years for CLL patients and 3.5 years for NHL patients. No death related to AC was recorded. Conclusions. B-cell CLL and NHL are associated with an increased risk of AC and ITP. The incidence of this AC is low. Although most of AC are diagnosed concurrently especially in advanced stages of the CLPD, sometimes they can precede the diagnosis of CLL or NHL. The majority of patients responded well to corticosteroid and/or chemotherapy. The outcome (therapeutic response, survival) and prognosis are similar to other patients with CLL or NHL.

DIFFERENT PROFILES OF ADHESION MOLECULES IN B-CELL NON-HODGKIN’S LYMPHOMA (B-NHL) ARE ASSOCIATED WITH PERIPHERAL BLOOD INVASION


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The frequency of the leukemic phase is different in the various histological subtypes of B-NHL. In chronic lymphocytic leukemia (CLL) the involvement of peripheral blood is always present; in mantle-cell lymphoma (MCL), the leukemic phase is present in 30%-70%; and in nodal and splenic marginal B-cell lymphoma (MZL) in about 10% and 50%, respectively. We hypothesized that the down-regulation of some adhesion markers could contribute to the leukemic dissemination observed in some B-NHL subtypes. We evaluated the expression of 10 adhesion molecules in tumor cells of peripheral blood of 17 patients with CLL, 17 with MCL, and 13 with nodal or splenic MZL, all in leukemic phase. All cases of CLL had 4 or 5 points in the Matutes scoring system, while MCL and MZL cases had between 0 and 3 points. In addition, all MCL cases had evidence of CYCLIN D1 overexpression. The mean fluorescence intensity (MFI) of the adhesion molecules in tumor cells was measured by flow cytometry in CD19-positive cells. The MFI of CD11a, CD11b, CD11c, CD18, CD49c, CD49d, CD29 and CD54 were different in the three groups (Table).
Table: Median values of M.F.I of adhesion molecules.

<table>
<thead>
<tr>
<th></th>
<th>CLL</th>
<th>MCL</th>
<th>MZL</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD11a</td>
<td>187.9</td>
<td>257.8</td>
<td>401.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CD11b</td>
<td>0</td>
<td>21.7</td>
<td>42.7</td>
<td>0.0011</td>
</tr>
<tr>
<td>CD18</td>
<td>48.5</td>
<td>0</td>
<td>143.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CD19</td>
<td>105.9</td>
<td>159.2</td>
<td>275.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CD49e</td>
<td>75.5</td>
<td>0</td>
<td>0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CD49d</td>
<td>97.9</td>
<td>249</td>
<td>320.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CD54</td>
<td>112.8</td>
<td>118.8</td>
<td>218</td>
<td>0.0010</td>
</tr>
<tr>
<td>CD44</td>
<td>311.4</td>
<td>316.4</td>
<td>285</td>
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<tr>
<td>CD54</td>
<td>273.4</td>
<td>233.8</td>
<td>317.7</td>
<td>0.0018</td>
</tr>
<tr>
<td>CD82</td>
<td>2</td>
<td>10.2</td>
<td>12.1</td>
<td>0.4965</td>
</tr>
</tbody>
</table>

*p*<sup>*</sup> Kruskal-Wallis test

The Dunn post test was applied when the p value was <0.05. The comparison between CLL and MCL showed that CLL presented a higher expression of CD11c and CD49c, and a lower expression of CD11b and CD18, CD49d, CD29 and CD54. Finally, the comparison between MCL and MZL showed that the MZL had a higher expression of CD11a, CD11c, CD18, CD29 and CD54. The structure of normal lymphoid follicle in lymph nodes depends on an appropriate association between the B-lymphocytes and the dendritic follicular cell through the interaction between CD11a and ICAM-1, as well as CD49d and VCAM-1. The lower expression of CD11a and CD49d on CLL cells could facilitate their detachment from the lymph node to invade the peripheral blood. A higher frequency of splenic involvement has been reported in cases of CLL with strong positivity to CD11c. However, in our series 82% of the MCL patients presented with an enlarged spleen, but showed the lowest expression of CD11c among the groups. Thus, our findings give support to the role of adhesion molecules in the determination of nodal or leukemic presentation in lymphoid malignancies.

1394 SAFETY AND EFFICACY OF A COMBINATION REGIMEN CONTAINING PENTOSTATIN, CHLORAMBUCIL AND METHYLPREDNISOLONE IN ELDERLY PATIENTS WITH PROGRESSING CHRONIC LYMPHOCYTIC LEUKEMIA

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**Background:** Chronic lymphocytic leukemia (CLL) is a neoplastic lymphocytic disorder most commonly seen in the elderly. Alkylating agents used to be the traditional first line treatment, however their inferior response rate and the inability to prolong survival have resulted in purine nucleoside analogues (PNAs) being used as first- and second-line therapy for patients with CLL. Management decisions are more difficult in the elderly because of the increase in toxicity of PNAs in this population. Pentostatin has been proven to be effective and less myelotoxic compared to other PNAs. We wished to evaluate the safety and efficacy of an alternative chemotherapeutic regimen containing pentostatin, chlorambucil and methylprednisolone in CLL patients with progressive disease. **Patients and Methods:** Five elderly, previously multiply treated CLL patients with progressive disease (5 male, 2 female, median age 75.5 years) and one patient with progressing Waldenstrom’s macroglobulinemia (WM) were enrolled in the study. Pentostatin was given intravenously at a dose of 4 mg/m², days 1 and 15, chlorambucil was given orally at a dose of 10 mg/d, days 1-7 and methylprednisolone orally 32 mg/d, days 1-7. The cycle was repeated every 30 days. All CLL patients had stage C disease (Binet system). **Results:** Four out of five CLL patients responded to treatment. Response was manifested as normalization of the full blood count and significant reduction in lymphadenopathy and/or organomegaly when previously present. **Response** was noted at the end of the second cycle. One patient died after the first cycle due to refractory disease. The patient with WM did not respond to treatment and developed grade IV neutropenia leading to discontinuation of this treatment. From the four responders one patient developed grade IV mucositis and delayed further courses and one patient had 3 febrile neutropenic episodes requiring admission. All patients developed at least grade III neutropenia and received G-CSF support. **Conclusions:** Combination therapy with pentostatin, alkylators and steroids seems to be active in CLL patients with progressive disease. However due to increased toxicity especially in the elderly, we suggest that pentostatin should be given at a lower dose. Addition of appropriate antibiotic, antifungal and antiviral therapy and G-CSF support is advisable in order to reduce infection risk in these patients.

1395 RISK OF CANCER IN PATIENTS WITH B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA. EXPERIENCE AND REPORT FROM A RETROSPECTIVE STUDY IN HEMATO-ONCOLOGY DEPARTMENT, UNIVERSITY HOSPITAL OLOMOUC

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**Background:** B-cell chronic lymphocytic leukemia (B-CLL) is characterised as a chronic indolent disease with an immunodeficiency. It is the most common leukemia of adult people, especially in the elderly. It is known that immunodeficiency and age can be the risk factors of cancer. Based on these facts we analyzed retrospectively our own data from patients with B-CLL who were diagnosed in our centre from 1994 to 2004. **Patients and Methods:** We analyzed group consisting of 245 patients, male/female 129/86. The median age of patients was 64 (35 - 91), in the clinical stage Binet A 123 (57%) patients, Binet B 55 (26%) patients, Binet C 37 (17%) patients. There were 19 patients who had a malignancy before the diagnosis of B-CLL was determined such as melanoma (4), breast cancer (3), colorectal cancer (2), basal cell carcinoma (1) and squamous cell carcinoma (1), uterus carcinoma (1), breast (1), lung (1), stomach (1), prostate (1), parotid gland (1), osteochondroma (1), leiomyosarkoma (1) and urinary bladder papilloma (1). Two patients had two of these tumors as listed above. There were 15 patients who developed second solid tumors after a diagnosis of B-CLL was established and 4 of them did not receive any chemotherapy for B-CLL. These second tumors involved basal cell carcinoma (3), colorectal carcinoma (2), cancer of the thyroid gland (2), lung (2), kidney (2), prostate (1), squamous cell carcinoma (1), uterus carcinoma (1) and bone metastasis (1). The incidence of all solid cancer was in 34 patients (16%), ratio male/female - 20/14 with a median age of 69. Most of these solid tumors were diagnosed in the clinical stage Binet A in 18 patients (53%), Binet B in 8 patients (24%), Binet C in 8 patients (24%). **Conclusion:** The development of second solid cancers in B-CLL diagnosed patients represents a high risk factor and a complication among long term survivors. Longer follow-up is needed to assess proper anamnesis, physical examination (skin lesion included) and differential diagnosis.

1396 MULTIPLE MYELOMA IN A PATIENT WITH CHRONIC LYMPHOCYTIC LEUKEMIA. EVIDENCE OF A COMMON PATHOLOGICAL CLONE

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The coexistence of chronic lymphocytic leukemia (CLL) and Multiple Myeloma (MM) in the same patient is very rare. It is uncertain whether the myeloma cell represents a clonal evolution of the CLL cell or a totally different cell population. We present one female, 62 years old patient suffering from CLL. From 1998 to 2005 she has received therapy for the CLL (chlorambucil and fludarabine in combination with cyclophosphamide). On March 2005 she was presented with pancytopenia and the diagnosis of Multiple Myeloma (MM) was established. In the bone marrow aspirate a diffused infiltration from 40% λ- monoclonal myeloma cells and 40% interstitial infiltration from CLL cells was found. The immunophenotype showed the same light chain in both the MM and CLL bone marrow cells examined. The G-banding conventional karyotype and the molecular cytogenetic analysis by M-FISH and M-BAND showed two different clones. One clone with 45 chromosomes and t(13;14)(p11:p11) and the other with the same chromosomal abnormality and additional complex chromosome rearrangements such as deletion of one chromosome 4, t(4;9), t(4;9;15), t(6;9;15), t(5;11), t(8;17), and t(16;21). The patient underwent chemotherapy with thalidomide plus dexamethasone and had a short partial remission of both diseases. Finally, she died on August 2005. Conclusions: The fact that the neoplastic cells carried the same light chain and the presence of translocation.
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IMMUNE THERAPY IN CHRONIC LYMPHOCYTIC LEUKEMIA: ALEMTUZUMAB (MABCAMPATH) (ANTI-CD52)
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Backgrounds. Chronic lymphocytic leukemia (CLL) is the most prevalent leukemia in adults in The Netherlands. Recent developments with monoclonal antibodies offer new therapeutic options. Alemtuzumab (MabCampath)® is a monoclonal antibody aimed at CD52, which is present on both normal and abnormal B and T lymphocytes. It is indicated as third line therapy in the treatment of CLL, after failure of conventional therapy including fludarabine. The most important side effects of Alemtuzumab treatment are opportunistic infections (pneumocystis carinii pneumonia (PCP) and CMV pneumonitis). It is unclear whether these complications do indeed lead to problems in the treatment of CLL patients in the Netherlands. Aims. To gain insight in the use and complications of Alemtuzumab in the Netherlands. Methods. A questionnaire concerning the treatment of CLL patients with Alemtuzumab was made on the basis of the literature [1] and sent to 11 hospitals in The Netherlands. Results. From 18-02-02 until 01-04-05 22 patients (mean age 64 years, 16 men, 6 women) with CLL RA/BIENET stage II A to IV were treated with 26 treatments of Alemtuzumab according to schedule (starting dosages 3, 10, 30 mg, followed by 3 times per week 30 mg i.v./s.c. for 4-12 weeks). Patients had received a mean of 3 lines of previous therapy before starting on Alemtuzumab. The time from diagnosis until the start of Alemtuzumab treatment was 5.6 years (4.5) (mean, (SD)). The duration of treatment was 9 (5.4) weeks (mean, (SD)). Reasons for early discontinuation of therapy were: fever and other side effects 20%, progressive disease (PD) 13%, complete response (CR) 13%, bone marrow toxicity 13%, other reasons 7%, unknown 35%. 27% of the treatments could be continued for the full 12 weeks. The most prevalent side effects were fever 78%, rigors 49%, dyspnea 19% and tiredness 15%. Infectious complications were pneumonia 26.9% (of which 1 PCP), sepsis 7.7%, herpes zoster 7.7%, sinustitis 7.7%, meningitis 3.8%, guillain barre 3.8%, others 15.3%. The response attained was CR 17%, partial response 35%, stable disease 30% and PD 17%. The duration of treatment was 9.5 (7) months (mean, (SD)). Summary/Conclusions. Treatment with Alemtuzumab is often discontinued prematurely. Therefore the maximal treatment effect cannot be reached. Fear of severe uncontrollable opportunistic infections seems unfounded.


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Chromosomal aberrations, detected by FISH, are considered as one of the most important prognostic factor in B CLL. Due to its expensive cost we chose to focus our analysis only on patients who required therapy. Fifty-five patients were studied, including 12 patients in stage Binet A and showing progressive disease. The lymphocytes were fixed for analysis before starting cytoreductive therapy. Examination included: 13q, 17p and 11q deletions and chromosome 12 trisomy. The results are presented in figure. The 17p deletions were found in 5 cases, including 2 active PLL patients and 3 CLL patients in stage Binet C. Trisomy 12 was found in 9 patients, all except one exhibited CLL/PLL or PLL morphology. The 11q deletions were found in 8 Binet C patients. The 13q deletion was found in 13 patients including 4 in stage Binet A, 5 in stage B, 3 in stage C and 1 with aggressive PLL.

In addition, 13q deletion was associated with 17p del (2 patients), 11q del (4 patients) and 12 trisomy (3 patients). Single case disclosed 12+11q del. Overall, chromosomal aberrations other than 13 del, were found in 4 out of 12 patients diagnosed in stage Binet A. FISH didn't show any aberrations in 15 cases. Considering the high prognostic significance of FISH analysis in CLL requiring therapy, it would be expected that patients in advanced stages and with progressive disease have unfavorable results. Nevertheless, our analysis in CLL patients requiring therapy showed that FISH results do not always correlate with the clinical stage of the disease. Part of the patients in stage Binet A had chromosomal aberrations supporting the need for therapy, but in other cases FISH revealed a favorable profile. Altogether, half of our patients disclosed either normal FISH results or the favorable 13q del. In conclusion, decision for treatment in patients with CLL cannot rely on FISH analysis alone and should be accompanied by additional prognostic factors.

References

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ANALYSIS OF RISK FACTORS OF 248 PATIENTS WITH B-CHRONIC LYMPHOCYTIC LEUKEMIA AT DIAGNOSIS
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248 patients (139 men and 109 women) with B-cell chronic lymphocytic leukemia were evaluated at diagnosis with respect of clinical stage, CD38 and ZAP-70 expression, cytogenetic changes (by FISH method) and IgVH mutation status and impact of these for overall survival. In Rai 0 and I clinical stage were 203 patients (82.9%), in stage II were 25 patients (10.2%) and in stage III and IV were 17 patients (7.0%). CD38 expression was evaluated at 137 patients (57.2%), positive was at 49 patients (35.8%), negative was at 88 (64.2%) patients. ZAP-70 expression was evaluated at 109 patients (44% from the total number), positive was at 44 patients (40.4%), negative at 65 (59.6%) patients. From 160 evaluations of IgVH mutation status (64.5% of the total number of patients) 71 (44.4%) patients were non-mutated and 89 (55.6%) cases were mutated. From 192 evaluated patients (77.4% of the total number of patients) trisomy of 12 chromosome was present at 22 patients (11.5%), one case was borderline (0.5%), 13q(14) deletion was present at 107 cases (56.6%) out of 189 evaluated (76.2% of the total number of patients), 11q(23) deletion was found at 4 cases (12.9%) out of 31 evaluated (12.5% of the total number of patients). In 13q(14) deletion was present at 13 cases (16.5%) out of 79 evaluated (31.9%) and 17(p13) deletion by 22 patients (11.4) out of 193 evaluated (77.8%). 48 patients died (19.4%), overall survival in 5 years was 83.5%, in 10 years 58.2%. According to our analysis sex (p=0,0002), Rai clinical stage (p=0,0002), IgVH mutation status (p=0,0001), 17(p13) deletion (p=0,09), CD38 (p=0,05) and ZAP-70 expression (p=0,02) revealed to be significant prognostic risk factors.

HALF OF CLL PATIENTS REQUIRING THERAPY DISCLOSE NORMAL FISH KARYOTYPE OR THE FAVORABLE 13q DELETION
Kaplan Medical Center, REHOVOT, Israel

Chromosomal aberrations, detected by FISH, are considered as one of the most important prognostic factor in B CLL. Due to its expensive cost we chose to focus our analysis only on patients who required therapy. Fifty-five patients were studied, including 12 patients in stage Binet A and showing progressive disease. The lymphocytes were fixed for analysis before starting cytoreductive therapy. Examination included: 13q, 17p and 11q deletions and chromosome 12 trisomy. The results are presented in figure. The 17p deletions were found in 5 cases, including 2 active PLL patients and 3 CLL patients in stage Binet C. Trisomy 12 was found in 9 patients, all except one exhibited CLL/PLL or PLL morphology. The 11q deletions were found in 8 Binet C patients. The 13q deletion was found in 13 patients including 4 in stage Binet A, 5 in stage B, 3 in stage C and 1 with aggressive PLL.

In addition, 13q deletion was associated with 17p del (2 patients), 11q del (4 patients) and 12 trisomy (3 patients). Single case disclosed 12+11q del. Overall, chromosomal aberrations other than 13 del, were found in 4 out of 12 patients diagnosed in stage Binet A. FISH didn't show any aberrations in 15 cases. Considering the high prognostic significance of FISH analysis in CLL requiring therapy, it would be expected that patients in advanced stages and with progressive disease have unfavorable results. Nevertheless, our analysis in CLL patients requiring therapy showed that FISH results do not always correlate with the clinical stage of the disease. Part of the patients in stage Binet A had chromosomal aberrations supporting the need for therapy, but in other cases FISH revealed a favorable profile. Altogether, half of our patients disclosed either normal FISH results or the favorable 13q del. In conclusion, decision for treatment in patients with CLL cannot rely on FISH analysis alone and should be accompanied by additional prognostic factors.

References

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**Rituximab (375 mg/m² d 3) every 28 days.** FIR was administered from more, as evidence of antigen selection is detected in both studies of the binding sites of restricted Ig, are necessary to elucidate skewed distribution of R mutations indicates that certain Ags may be selected. Also one

**1402**


**1403**

**Impact of Trisomy 12, Del(13q), Del(17p) and Del(11q) on the Immunophenotype, DNA Ploidy Status and Proliferative Rate of Leukemic B-Cells in Chronic Lymphocytic Leukemia**


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**1401**

**FLUDARABINE-IFOSFAMIDE-RITUXIMAB (FIR): A THERAPEUTIC OPTION FOR CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) PATIENTS RESISTANT TO CHLORAMBUCIL**

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Ifosamide is an alkylator belonging to the oxazaphosphorines whose efficacy, alone or in combination as mobilizing agent or as salvage therapy, has been extensively documented in solid tumours and in lymphomas, but not yet in CLL. Fludarabine is effective in CLL patients refractory/resistant after chlorambucil and its effectiveness in this disease is enhanced by the association with cyclophosphamide (FC) and monoclonal antibodies, in particular Rituximab (FCR combination). To evaluate the efficacy and safety of a Fludarabine Ifosamide and Rituximab-containing regimen in a series of CLL patients refractory/resistant to chlorambucil, FIR schedule: Ifosamide (750 mg/m² dd 1-3), Fludarabine (25 mg/m² dd 1-3) and Rituximab (375 mg/m² d 3) every 28 days. FIR was administered from March 2002 to April 2005 within a monocentric phase II trial to 14 patients with advanced CLL (sex: M/F=9/5; age: median 63 yrs; range 51-72 yrs; IWGCLL Stage: C in 3 patients, B in 10 patients and A in 1 patient in progression). All patients were refractory/resistant to alkylators and 50% of them have also experienced Fludarabine; 1 patient has also received Rituximab (previous lines of therapy median: 2, range: 1-2). Patients received a median of 4 FIR cycles (range 1-6). Oral trimethoprim/sulfamethoxazole and acyclovir prophylaxis was given to all the patients during FIR therapy, and two months thereafter. Twelve out of the 14 enrolled patients completed at least 3 FIR cycles. Response was evaluated according to the NCI Working Group criteria on an intention to treat population of 14 patients. An overall response rate (ORR) of 64.2% was achieved, including 7.2% complete response (CR) and 57% partial responses (PR). Stable disease was documented in 25% of patients and no progression on therapy was observed. Responses according to site were as follows: peripheral blood 78.6% (CR 63.3%), lymph node 64.3% (CR 35.7%), bone marrow 78.6% (CR 14.3% PR 64.5%). Early death was observed in 2 patients (one septic shock during therapy-related neutropenia and one cerebral hemorrhage, respectively 10 and 60 days after start of FIR therapy). Grade 3 WHO haematological toxicity was documented in 78% of patients (neutropenia 57%), whereas non-haematologic toxicity was documented in 57% (infection 3 cases, gastrointestinal 3 cases, cutaneous 1 case, renal 1 case). Three out of nine responders have relapsed to date (1 PR after 14 months, 2 PR at 15 and 19 months, respectively). Response is maintained in 5-12 months follow-up of 25 months (range 0.5-48 months). Overall survival of non-responders was 11 months (range 0.5-47 months), whereas it was not reached for responders, after a median follow-up of 32 months (range 11-48 months). These preliminary data, obtained in a subset of advanced and pretreated CLL patients, showing a sustained and durable response rate with an acceptable incidence of adverse events, suggest that a chemotherapeutic regimen combining Fludarabine, Ifosamide and Rituximab may be a feasible therapeutic alternative in patients affected by relapsing and refractory CLL.

**IMPACT OF TRISOMY 12, DEL(13Q), DEL(17P) AND DEL(11Q) ON THE IMMUNOPHENOTYPE, DNA PLOIDY STATUS AND PROLIFERATIVE RATE OF LEUKEMIC B-CELLS IN CHRONIC LYMPHOCYTIC LEUKEMIA**


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B-cell chronic lymphocytic leukemia (B-CLL) is a well-defined clinical entity which displays a variable clinical course in association with the existence of heterogeneous molecular and cytogenetic features. To analyze the relationship between the presence of trisomy 12, 13q-, 17p- and 11q- and the immunophenotype, DNA ploidy status and proliferative rate of leukemic B-Cells in Chronic Lymphocytic Leukemia

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tive characteristics of neoplastic B-CLL cells. The impact of trisomy 12, del(13q), del(17p) and del(11q) was determined by interphase fluorescence in situ hybridization analysis (ISH) of purified neoplastic B-cells from a series of 180 patients with newly diagnosed B-CLL on the immunophenotype, DNA ploidy status and the proliferative rate of neoplastic B-cells. Half (50%) of all B-CLL cases studied displayed one (40%) or more (10%) of the genetic abnormalities, trisomy 12 and del(13q) being the most frequently detected ones (25% and 21%, respectively), del(17p) and del(11q) being found in 9% and 4.9% of the cases, respectively. Trisomy 12 was associated with a higher frequency of DNA aneuploidy (p=0.012) together with a higher reactivity for CD22, CD27, CD24 and CD79b. The expression of this latter marker was also higher among cases with 17p- which in turn showed reduced CD11c expression. Cases carrying del(13q) showed a higher expression of CD5, CD43 and CyBCL2, these latter two markers being also brighter among cases with 11q-. Remarkably, none of the chromosomal abnormalities investigated was associated with an increased proliferation of neoplastic B-cell by itself, although B-CLL cases simultaneous showing 13q- and 17p- displayed a higher percentage of S/G2/M-phase tumor cells as compared with individuals carrying either 13q- (p=0.02) or 17p- cases showing no genetic abnormalities (p=0.03). In summary, our results confirm and extend previous observations about the frequency of trisomy 12, 13q-, 17p- and 11q- in B-CLL patients, where they affect only a variable proportion of all neoplastic cells showing that the abnormalities detected in these cases may have an impact on the immune regulation of B-CLL cells; in contrast, the impact of these cytogenetic abnormalities on the proliferative rate of neoplastic B-cells was only noted for cases simultaneously carrying 13q- and 17p-.

1404 ANGIogenic cytokines in B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA: ASSOCIATION WITH IgVH MUTATION STATUS AND GENETIC ABNORMALITIES

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Backgrounds. B-cell chronic lymphocytic leukemia (B-CLL) is a disease with an extremely variable clinical course. New prognostic factors such as mutation status of immunoglobulin light chain variable regions (IgVH) or genetic aberrations detected by fluorescent in situ hybridization (ISH) are being increasingly used in order to identify patients with high-risk disease. Several studies have shown that angio genesis is increased in B-CLL and may potentially help in prognostic assessment of B-CLL patients. Aim. To assess relationship between plasma concentrations of vascular endothelial growth factor (VEGF) or basic fibroblast growth factor (bFGF) and IgVH mutational status or genetic aberrations. Methods. We measured VEGF and bFGF using sandwich enzyme-linked immunosorbent assay (ELISA) kits in peripheral blood plasma of 49 patients (males, females, age) with untreated B-CLL and 50 healthy donors. IgVH mutation status was analyzed using cDNA transcribed from B-CLL RNA for touch-down reverse transcriptase polymerase chain reaction (RT-PCR) with degenerate primers for VHI-7 families; IgVH sequences were aligned to the nearest germline using the Ig BLAST database. There were 26 patients with low risk, 17 with intermediate risk and 4 with high risk disease. The disease status was categorized as follows: Mutated IgVH genes (i.e. more than 2% of somatic mutations) were identified in 23 and unmaturated in 26 patients. Genetic abnormalities using fluorescence in situ hybridization (ISH) probes for del13q, del11q, del17p and +12 were investigated in 40 patients. We divided patients according to genetic aberrations into favourable (no abnormalities) or high-risk status. Results. There was a statistically significant increase of both VEGF (p=0.006) and bFGF (p<0.0001) in patients with B-CLL compared to the control group. Patients with mutated IgVH genes had significantly higher concentrations of bFGF (p=0.0149) but not VEGF (p=0.146) than those with unmaturated IgVH. Furthermore, bFGF was significantly higher in both IgVH subgroups (p<0.0001) while VEGF was significantly elevated in IgVH mutated (p=0.0002) but not unmaturated patients (p=0.0788). Regarding cytogenetics, significant difference between patients with favourable vs. unfavourable aberrations was nei ther in VEGF nor bFGF levels (p=0.878 and p=0.494). Conclusions. This study confirms what angiogenic activators are elevated in patients with B-CLL. Interestingly, bFGF but not VEGF was significantly higher in patients with mutated IgVH genes in comparison to unmaturated IgVH. We did not observe significant difference between low- and high-risk genetic abnormalities. The data must however be interpreted with caution due to relatively low number of patient. Larger study is necessary to perform a more detailed statistical assessment including multivariate analysis.

1405 CASE REPORT: PLASMA CELL LEUKEMIA SUCCESSFULLY TREATED WITH BORTEZOMIB, MELPHALAN, PREDSINONE AND THALIDOMIDE (H-M PT)

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Plasma cell leukemia (PCL) is an aggressive disease defined as circulating peripheral blood plasma cells exceeding 2×10^9/L or 20% of peripheral blood plasma cells (1). The disease can occur as primary disease or as part of a disease entity of a Multiple Myeloma (2). The disease is very aggressive with median overall survival of about seven months for primary and 2 months for secondary plasma cell leukemia (2). This is the report of a 67 year old woman with three years history of MGUS (monoclonal gammopathy of unknown significance) IgG lambda that evolved into Multiple Myeloma stage IIA according to Salmon and Durie (3). The patient was treated using induction chemotherapy (4 cycles of Vin-cristine, Doxorubicine and Dexamethasone (VAD)), stem cell mobilisation with IV (Ilosfamide, Epirubicine, Etoposide) and double autolo- gous transplantation after conditioning with Melphalan 140mg/m2. After the second autologous transplantation the patient remained in partial remission showing a small monoclonal component in the serum for seven months when the patient presented at the day hospital with diffuse effusions. Peripheral blood showed 19×10^9/L white blood cells, 8.9 g/dL of hemoglobin and 16×10^9/L platelets with a differential leukocyte count of 50% neutrophils, 22% lymphocytes, 4% monocytes and 24% plas macells. Flow cytometric analysis confirmed the presence of 56% plasmacells as shown by the expression of CD138. All of them had an aberrant antigen expression (CD138/CD19/CD56). Further the patient had acute renal failure with a creatine-level of 4.1g/dL. The monoclonal component had risen to 5g/L. Bone marrow aspiration was not possible (puncto sicca) and histological examination showed an almost complete infiltration of the bone marrow by clonal plasmacells of intermediate differentiation. The patient was initially treated with dexamethasmone 40 mg/die on days 1-4, 9-12 and 17-20 for two cycles with reduc tion of plasmacell infiltration in the bone marrow to 50% of all nucleated cells. After clearance of plasmacells in the peripheral blood smear every 35 days, the patient was monitored at three-month intervals. The patient was treated using induction chemotherapy including bortezomib (1.3 m2 on days 1, 4, 8, 11), thalidomide (50mg/die) and double autologous transplantation after conditioning with Melphalan 140mg/m2. After the second autologous transplantation the patient remained in partial remission showing a small monoclonal component in the serum for seven months when the patient presented at the day hospital with diffuse effusions. Peripheral blood showed 19×10^9/L white blood cells, 8.9 g/dL of hemoglobin and 16×10^9/L platelets with a differential leukocyte count of 50% neutrophils, 22% lymphocytes, 4% monocytes and 24% plasmacells. Flow cytometric analysis confirmed the presence of 56% plasmacells as shown by the expression of CD138. All of them had an aberrant antigen expression (CD138/CD19/CD56). Further the patient had acute renal failure with a creatine-level of 4.1g/dL. The monoclonal component had risen to 5g/L. Bone marrow aspiration was not possible (puncto sicca) and histological examination showed an almost complete infiltration of the bone marrow by clonal plasmacells of intermediate differentiation. The patient was initially treated with dexamethasmone 40 mg/die on days 1-4, 9-12 and 17-20 for two cycles with reduction of plasmacell infiltration in the bone marrow to 50% of all nucleated cells. After clearance of plasmacells in the peripheral blood smear every 35 days, the patient was monitored at three-month intervals. The patient was treated using induction chemotherapy including bortezomib (1.3 m2 on days 1, 4, 8, 11), thalidomide (50mg/die), dexamethasone (40mg/m2 on days 1-5) (5) was started. After three of four cycles (recy cling every 35 days) the patient had a normal peripheral blood count and the monoclonal component in the peripheral blood disappeared while immunofixation remained positive (near complete Remission nCR) A maintenance therapy with daily thalidomide (50mg/die) and dexam ethasone (40mg/m2 on days 1-5) was continued every 28 days for 8 cycles and eleven months after diagnosis of plasmacell leukaemia the patient is still in nCR without significant side effects. Combination therapy including bortezomib, thalidomide, melphalan and prednisone should be consid ered as effective and safe treatment of plasmacell leukaemia.

1406 MAINTENANCE WITH VERY LOW DOSE THALIDOMIDE AFTER AUTO-SCT IN MULTIPLE MYELOMA: LOW TOXICITY AND IMPROVED OUTCOME

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High dose therapy with single or double transplantation (auto-SCT) has improved prognosis of multiple myeloma (MM). New drugs are
promising in upfront therapy while the role of maintenance is still debated. Thalidomide (thal) is an active drug in the treatment of myeloma, and is being investigated as first line therapy. It could be useful in the control of minimal residual disease. We used thal as maintenance after autologous transplantation (single or double) and compare the outcome with other maintenance or none. From January 2001 to December 2005 25 patients (15 males and 12 females) with MM have been treated in our institution. Median age was 58 years (range 40–72), 12 were IgG, 7 IgA, 4 light chains and 2 plasma-cell leukaemia. Treatment was 4 cycles of VAD regimen followed by auto-SCT. 9/25 performed double auto-SCT. 3 months after SCT, 13 patients (9 single and 4 double SCT) began thal 50 mg/die as maintenance therapy. 12 patients (7 single and 5 double SCT) received IFN-γ (4/13), dexametasone (8/15) or no therapy (5/13). The 2 groups were regarding the type of myeloma: 6 IgG, 3 IgA, 3 light chains and 1 plasma-cell leukaemia in the thal group; 5 IgG, 4 IgA, 1 IgD and 1 plasma-cell leukaemia in the other. Response to SCT: 3 CR, 7 PR and 1 NR in the thal group, 5 CR, 4 PR and 1 NR in the other. In the thal group 3/11 patient relapsed. Median follow up from the beginning of maintenance therapy was 30 months (range 9–46) with 7/10 patients in CR or stable disease, with a progression free survival (PFS) and overall survival (OS) projected at 47 months of 73%. In the other group, 8/10 patients relapsed. Median follow up was 30 months (range 4–54) with a median PFS and OS of 8 and 18 months respectively. In the two groups the patients in progression have effected, as lifesaving therapy, thalidomide 200 mg / die + dexametasone 20 mg days gg 1–4 and 15–18. The difference between the 2 groups is statistically significant for PFS (p: 0.007), and not significant for OS (p: 0.057) even if difference (73% vs. 10% at 100 months) appears clear (Graph 3). From diagnosis the median OS is of 72 months in no thal group and is 75% projected at 100 months (p: 0.2 graph 3) in the thal group. Thalidomide was administered for a median period of 12 months, being neurological toxicity the main reason of suspension (3/10 patients). Neurological toxicity grade I–III was present in 65% of patients, while haematological toxicity grade 1 occur in 55% of patients. In conclusion, in a small number of patients low dose thal as maintenance after auto-SCT resulted in an improved PFS and OS when compared with other or none maintenance, with acceptable toxicity. Further studies in larger cohorts and randomized trials are needed to confirm this experience.

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**Backgrounds.** The correlation between the activity of angiogenesis (microvessel density and VEGF-A expression) and certain clinical characteristics (such as progression and poor prognosis) for patients with MM is shown. VEGF-A is known as the main tumor angiogenic factor, but several other factors of VEGF family (such as VEGF-C and VEGF-D) could play a role in stimulation of angiogenesis. As compared to VEGF-A, the expression of VEGF-C and VEGF-D, as well as their possible role in MM progression is much less studied. The study of VEGF-A, as well as other growth factors of VEGF family, and their receptors gene expression in patients with MM can bring to discovery of the new proangiogenic target. **Aims:** To investigate the VEGFs (VEGF-A, VEGF-C and VEGF-D) and their receptors (VEGFR1, VEGFR2 and VEGFR3) genes expression in bone marrow aspirates of MM patients. To compare the intensity of gene expression with such clinical characteristics as disease progression and resistance to therapy. **Methods:** Mononuclear cell (MNC) fraction was purified from the bone marrow aspirate of patients with newly diagnosed MM by Ficoll-Hypaque centrifugation. Gene expression was evaluated by semi-quantitative RT-PCR technique. **Results:** The gene expression was studied in bone marrow aspirates of 13 patients with newly diagnosed MM, III stage, aged between 50 and 73 years. These patients had the following immunochemical types of MM: 7 patients - IgG, 1 patient - IgA, 1 patient - Ig M, 2 patients with Myeloma Benh-Johns, 2 patients had undefined type of MM. All patients had undergone cytostatic therapy according to M-2 protocol. VEGF-A was expressed in 12 patients, 6 of them displayed the high level of expression. One patient showed no expression. VEGF-A expression was absent in MNC of 1 patient, while 12 patients expressed VEGF-C mRNA and the high level of expression was observed in 5 patients. VEGF-D expression was registered in 8 patients (1 of them had high level of expression) and 5 patients were VEGF-D-negative. The intensive VEGFR1 expression was noticed in all 13 patients. VEGFR2 was expressed in one patient only. VEGFR3 was expressed in 10/13 patients with high levels of expression in 7 patients. The gene expression was compared with the severity of SWOG stage at as SCT. With median follow up of 17 months, median overall survival (OS) was 39 months. IgD MM had the lowest survival among the MM subtypes (p<0.01). Median OS of IgD MM, IgG MM, IgA MM, and free light chain MM were 12 months, 62 months, 40 months, and 55 months, respectively. Kaplan-Meier survival curve according to MM subtype was as Figure 1. **Summary/conclusion:** In this small-sized single center study from Korea, IgD MM had a poorer survival than other subtype after SCT.
the clinical symptom manifestation. The only patient with intensive VE), VEGF2, VEGFR1 and VEGFR3 expression, as well. Two other patients, who died during 8-12 months of disease progression, also had the high levels of VEGFs and VEGFRs expression. The comparison of VEGFs and VEGFRs expression in patients before and after therapy revealed that VEGF-C expression was stimulated in patients resistant to therapy. Conclusions: Our data showed the high frequency and the similar pattern of VEGF-A and VEGF-C expression in bone marrow aspirates of MM patients studied. VEGF-D and VEGFR3 were expressed to the lesser extent. The high level of VEGFR1 expression was registered in all patients, while all patients except one were VEGFR2-negative. Our data suggest that VEGF-C expression could be the predictor of poor prognosis.

1409 UNUSUAL CNS AND CUTANEOUS INVOLVEMENTS IN MULTIPLE MYELOMA DURING BORTEZOMIB TREATMENT

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Background. Since last years the proteasome has emerged as a real and exciting target for anticancer therapy. Velcade (bortezomib, PS341) remains the first selective proteasome inhibitor that has demonstrated significant preclinical activity in several tumor models and significant efficacy in patients with refractory or relapsed multiple myeloma. The major biological effect of bortezomib is the inhibition of the nuclear transcription factor NFkappab, with subsequent inhibition of the tumor cells growth, induction of apoptosis, inhibition of angiogenesis and of cellular adhesion. In vitro bortezomib induces apoptosis of multiple myeloma cells and inhibits cell adhesion within the bone marrow microenvironment. The preliminary results of several phase I and II studies showed high antmyeloma activity of bortezomib alone or in combination with cytotoxic agents such as doxorubicin, melphalan, dexamethasone or thalidomide in patients with newly diagnosed multiple myeloma.

Methods. We describe two cases of multiple myeloma patients that developed unusual cutaneous and CNS localizations during treatment with bortezomib alone. A 75 years old male patient with immunoglobulin G-kappa multiple myeloma resistant to previous therapies with melphalan and thalidomide received bortezomib (given day 1, 4, 8, 11 at 1.3 mg/m² every 21 days). After two courses a sierologic response (from IgG 7170 mg/dl to IgG 1560 mg/dl) was obtained. However, several cutaneous lesions localized at the face, arms and chest were presented. The histologic evaluation confirmed plasma cells localization, therefore dexamethasone was added to bortezomib and a complete disappearance was observed two weeks later. A 74 years old female patient with immunoglobulin G-lambda multiple myeloma resistant to previous treatments with melphalan and thalidomide started a treatment with bortezomib (given day 1, 4, 8, 11 at 1.3 mg/m² every 21 days) because of disease progression (IgG 3400). After the first course of bortezomib, while the monoclonal component drastically reduced (IgG 1470 mg/dl), multiple sub-cutaneous nodular lesions and meningeal involvement of multiple myeloma with massive infiltration of cerebellum appeared and the patient died after one week. Conclusions. To our knowledge, these are the first cases of cutaneous and CNS localizations of multiple myeloma during the treatment with bortezomib. Pharmacokinetic studies have demonstrated that after administration of a single dose bortezomib is rapidly distributed into nearly all tissues, with the exception of adipose tissue and certain tissues in the brain protected by the blood-brain barrier. Our case shows that bortezomib is fastly effective in reducing the size of the disease, but that it can’t pass the emato-tenceral barrier and can’t reach adipose tissue. Interestingly both patients were previously treated with thalidomide, a molecule that has been recently associated with extramedullary relapses probably because it increases the expression of cytoadhesion molecules such as CD138 and CD56 in myeloma cells. It could be of interest to evaluate in further studies the expression of cytoadhesion molecules also during treatment with bortezomib.

Figure 1. Patient 2. Cranial leptomeningeal myelomatosis.

1410 COMBINED ADMINISTRATION OF BORTEZOMIB AND DEXAMETHASONE IN THE TREATMENT OF REFRACTORY MULTIPLE MYELOMA (MM)

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Multiple myeloma (MM) is a neoplastic disease especially affecting elderly patients even if in recent years it has been also observed in younger patients. The use of the proteasome inhibitor bortezomib has been recently introduced in the treatment of relapsed and/or refractory MM. In fact, Bortezomib has proven to be safe and effective in MM patients not only as monotherapy but also given in combination with cytotoxic agents. Bortezomib-based combination regimens have induced clinical benefits with manageable toxicities and may ultimately lead to improvement in the duration of response and survival of patients in the first-line setting. In our institution we are following 45 patients with stage II/III MM and 7 out of 55 (4 F and 3 M, median age: 71 years, r.: 68-77 years) suspended chemotherapy after 6 cycles of Melphalan and Prednisone regimen for excessive toxicity even if they presented progression disease (PD) at the clinical re-staging performed with both serum marker evaluation and cytological examination of bone marrow blood. All the 7 patients refused thalidomide treatment and underwent a treatment with bortezomib (1.3 mg/m² i.v. day 1, 4, 8, 11 every 21 days) together with dexamethasone (40 mg i.v. days 1-4 every 21 days). At a clinical re-staging performed after four courses from the beginning of bortezomib-dexamethasone combined administration a partial remission (reduction of M-component > 50-75%) was recorded in 6 out of 7 patients while the remaining was in steady disease (SD). Thereafter all patients received further four courses of therapy. One month from the end of treatment two of seven patients achieved a complete remission (negative immunofixation) and the remaining showed a partial remission (PR). At the present, (month +9) only one patient shows a progression disease, while two patients are in CR and four in PR. Our results suggest that the combination of bortezomib and dexamethasone is effective and well tolerated even in elderly patients. Although there are several published data on the activity of the therapy based on the combination between bortezomib and dexamethasone, little is still known about the improvement in the duration of response and survival of elderly patients in the first or second line therapies.

1411 CLINICAL EFFICIENCY AND TOXICITY OF REGIMENS VAD AND HYPERCVAD IN MULTIPLE MYELOMA

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The primary objective of this investigation was to test the overall response rates and toxicity of the standard regimen VAD-D-D and
regimen HyperCVAD for first line treatment patients with multiple myeloma. First group consist of 40 patients (57±4 years) which received 4-6 standard regimens VAD-D-D every 28 days. Second group consist of 20 patients (57±3 years) which received 4-6 standard regimens HyperCVAD (Cyclophosphamide 300 mg/m² i.v. twice 1-3 days, vincristine 2 mg i.v. day 1 and 9, doxorubicine 50 mg/m² i.v. day 4, dexametazone 40 mg per os every 28 days) every 28 days. All patients had poor performance status, elevated LDH values, but did not have low platelet count. An objective response (complete or partial) was documented in 45% and 65% of patients treated with VAD and HyperCVAD, respectively. Hematological and non-hematological toxicities were mild or moderate and equally distributed between the two treatment arms with the exception of neutropenia III-IV, which was more common after HyperCVAD (90%). The duration of neutropenia was from 5 to 9 days. 3 (15%) patients had febrile neutropenia. Early death was in first and second groups 5% and 0% respectively. Project 3 years overall survival was 65% and 90% in VAD and HyperCVAD groups respectively. These preliminary data suggest that regimen HyperCVAD more effective than standard regimen VAD.

**1412 BORTEZOMIB AS SALVAGE TREATMENT IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA**


**Backgrounds.** Bortezomib is a proteasome inhibitor with significant antitumor activity, exerted through disruption of critical signaling cascades that promote cell adhesion and cycle progression, thus inducing apoptosis and decreasing cell proliferation. Bortezomib alone or in combination with dexamethasone has already been reported to yield high response rates and durable remissions in patients with relapsed/refractory multiple myeloma (MM). **Aim:** To evaluate the efficacy and safety of bortezomib as salvage treatment in our patients with relapsed/refractory MM. **Methods.** Between August 2004 and December 2005, 22 patients with progressive MM, 13 (59.1%) males and 9 (40.9%) females, of median age 65 (49-82) years, were treated with bortezomib. Thirteen (59.1%) patients were in relapse and 9 (40.9%) in refractory relapse. Median time from diagnosis was 22 (3-180) months. Patients had previously received a median number of 2 (1-4) different regimens and 5 (22.7%) had undergone high dose therapy with autologous stem cell transplantation. Bortezomib was administered to patients at a dose of 1.3 mg/m² on days 1, 4, 8 and 11 every 3 weeks. In case of stable or progressive disease after 2 therapy cycles, dexamethasone 20 mg PO was added to the regimen on days 1-2, 4-5, 8-9 and 11-12 of each cycle. Response and toxicity were evaluated according to the International Myeloma Working Group criteria. Remission duration (RD), overall survival (OS) and event-free survival (EFS) were estimated according to the Kaplan-Meier method. **Results.** Median follow-up time was 7 (2-15) months. Patients received a median number of 6 (1-8) therapy cycles. Median time-to-response was 2 (1-5) months. Complete response was observed in 2 (9.1%) patients, very good partial response in 4 (18.2%) and partial response in 11 (50%), yielding an overall response rate of 72.5%. Eight (36.4%) patients are dead and 14 (63.6%) are alive, 12 (85.7%) of which, in remission. Median RD and OS are not reached. Actuarial RD rate at 6 months and OS rate at 12 months was 57.6% and 52.5% respectively. Median EFS was 6 (95% CI: 1-15) months. Peripheral neuropathy was observed in 18 (59.1%) patients, thrombocytopenia in 11 (50%), fever in 9 (40.9%), microbial respiratory infections in 8 (36.4%), herpetic skin infections in 4 (18.2%), skin rash in 4 (18.2%), diaphoresis in 3 (13.6%), nausea/vomiting in 3 (13.6%), hypertension in 2 (9.1%) and constipation in 2 (9.1%). Grade III-IV peripheral neuropathy, thrombocytopenia and respiratory infections were observed in 3 (15.6%), 2 (9.1%) and 3 (15.6%) patients respectively. A case of grand mal seizure during bortezomib infusion was noted. **Conclusion:** Bortezomib proved in our study to be an effective regimen in the treatment of relapsed/refractory MM, yielding very high response rates, while toxicity was acceptable. However, EFS was short, though a longer follow-up may be required in order to estimate patients’ outcome more accurately.

**1413 DECREASED γδ T CELL RECEPTOR (TCR Gammadelta) EXPRESSION IN PERIPHERAL BLOOD LYMPHOCYTE POPULATION AND REDUCED SERUM OSTEOPROTEGERIN CONCENTRATION AS A TUMOUR ADVANTAGE MARKERS IN MULTIPLE MYELOMA (MM) PATIENTS**

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**Backgrounds.** γδ T lymphocytes (γδ T) appear to posses intrinsic cytolytic activity against tumour cells in carcinomas, sarcomas and lymphomas. OPG is known as natural inhibitor of osteoclastogenesis. **Aim:** To determine a mean percentage (%) of γδ T cells in peripheral blood and serum osteoprotegerin (OPG) concentrations of untreated MM patients (pts) and verify the impact of peripheral blood γδ T cells presence and serum OPG levels at the time of diagnosis on MM clinical advantage. **Material and Methods.** 25 newly MM pts, admitted to Department of Hematology, Blood Neoplasms and Bone Marrow Transplantation Project 2002-2005 were included into analysis. According to Case bone disease staging system: 6 pts were of stage 1, 4 of stage 2 and 15 of stage 3. Samples of blood and sera were taken at the time of MM diagnosis. γδ T cells were estimated by flow-cytometry (FACS), using a fluorescence-activated cell sorter and monoclonal antibodies (MoAbs: Abs-anti TCRγδ-1 FITC (Becton-Dickinson), Abs-anti CD14-PE/CD45-FITC (Leukogate) and CD3-PE). Serum OPG was estimated in enzyme-linked immunosorbent assay (ELISA, Biomedica GmbH, Wien, Austria). Results. In 12 of MM pts in 3 bone stage groups (group I) γδ T cells percentage, contained in interval 0,5 - 7,2 (mean = 2,75, SD = 1,9) was significantly (p<0,01) decreased as compared with 13 of MM pts in 0±1 bone disease stages (group II): 1,4 - 7,4 (mean=6,9, SD = 4,75). Despite a lack of statistical significance, the favorable trend was observed that serum osteoporogen (OPG) concentrations in MM patients with abundant bone involvement (group I) fluctuated from 0,9 to 5,3 pmol/ml (mean = 2,54, SD = 1,47) and was also lower than in MM pts with less advanced bone destruction (group I): 1,4 - 7,4 pmol/ml (mean = 3,68, SD = 2,07) (p=0,08). Moreover, possitive correlation between peripheral blood γδ T cell percentage at the time of diagnosis and serum OPG concentration was found: r = 0,48 (p=0,05). Conclusions. In MM decreased γδ T cell percentage in peripheral blood and reduced serum osteoprotein concentration, measured at the time of diagnosis seems to be advanced tumour markers in clinical practice.

**1414 CARDIAC AMYLOID DEPOSITION AS CAUSE OF MYOCARDIAL DYSFUNCTION IN MULTIPLE MYELOMA**


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**Backgrounds.** Amyloidosis (AL) occurs in 10-20% of patients with multiple myeloma (MM). Around 40% of them manifest cardiac involvement on echocardiogram, in only half of which, overt congestive heart failure is observed at presentation. Dopppl echochardiography best assesses myocardial function in AL by demonstrating inflow restriction during diastole. The precursor of B-type natriuretic peptide (pro-BNP) is known as sensitive biomarker secreted by the muscle cells of left ventricle (LV) in response to ventricular stress allowing early detection of myocardial dysfunction. **Aim.** To study the correlation between the extent of amyloid infiltration of myocardial wall, as expressed by the thickness of interventricular septum (IVS) and the degree of myocardial dysfunction, as translated by the value of E/A wave ratio on Doppler echocardiogram and the serum levels of pro-BNP. **Methods.** Twenty-six patients with multiple myeloma and no other medical condition that could affect the thickness of IVS or myocardial function, entered the study and were divided into Groups A and B according to the thickness of IVS (>14 mm and ≤14 mm respectively). Eighteen healthy individuals with thickness of IVS <11 mm, were used as control Group. **Results.** Dopppl echocardiography was performed in order to estimate the thickness of IVS and E/A wave ratio and blood was drawn in order to measure pro-BNP serum levels. One-way ANOVA tests were used to test the differences in the values of E/A wave ratio and the measurements of pro-BNP serum levels between groups. Differences were assessed using the long-rank test. Results. Group A included 15 patients, 10 males and 5 females of medi-
an age 54 years (43-67). Group B 11 patients, 5 males and 6 females of median age 50 years (41-68) and Group C 18 patients, 12 males and 6 females of median age 48 years (39-57). The mean values of E/A wave ratio in Groups A, B and C were 0.7, 0.92 and 1.12 respectively with statistically significant difference (p<0.01) among all groups. The mean serum pro-BNP levels in Groups A, B and C were 679 pg/ml, 384 pg/ml and 85 pg/ml respectively, also with statistically significant difference (p=0.001). Conclusion. Cardiac amyloid deposition in MM patients is responsible for LV stress and myocardial dysfunction, the degree of which correlates to the extent of myocardial amyloid infiltration. Doppler echocardiography and measurement of pro-BNP levels can be used for early detection of subclinical myocardial damage, before the latter evolves into overt heart failure.

**1415 HIGH INCIDENCE OF OSTEONECROSIS OF JAW IN PATIENTS WITH MULTIPLE MYELOMA TREATED WITH Zoledronic ACID**


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**Background.** Bisphosphonates are of proven value in the prevention of skeletal-related events in patients with multiple myeloma and metastatic bone disease. In multiple myeloma these are used routinely in patients with skeletal lytic lesions. Several classes of bisphosphonates are used, however recently the use of zoledronic acid has increased due to its high potency in preventing bone mineral loss. This has however been associated with a new oral complication, osteonecrosis of jaw, in a proportion of patients with myeloma. The pathogenetic mechanism is thought to be bisphosphonate induced increased osteoclast activity and reduced blood flow in the bone which delays bone healing. We evaluated in our institution all patients of multiple myeloma and metastatic cancer who have been treated with intravenous bisphosphonates and looked in the incidence of osteonecrosis of jaw, underlying predisposing factors and outcome after management of these patients. Aims. To look for the incidence, clinical presentation, underlying predisposing factors for the development of osteonecrosis of jaw in patients with multiple myeloma and metastatic cancer treated with intravenous bisphosphonates. To evaluate the treatment outcome and formulate guidelines for the prevention of this complication. Methods. We evaluated all patients of multiple myeloma and metastatic cancer treated in our Institution with intravenous bisphosphonates for the occurrence of osteonecrosis of jaw. We looked at the type of bisphosphonate used, duration of use, concurrent use of chemotherapy, clinical presentation, underlying predisposing factors and the treatment outcome. The suspected patients were evaluated and managed at the oral surgery department. Results. We found out of a total of 26 patients with multiple myeloma who were on intravenous bisphosphonates 6 developed pathologically proven osteonecrosis of jaw. Interestingly all six patients were females and all of them have received zoledronic acid. None of the 36 patients with non-haematological cancer who had received intravenous bisphosphonates developed osteonecrosis of jaw. The patient profile of the myeloma patients who developed osteonecrosis is given in table 1. Mean age of patients was 62 years. Median duration of onset of symptoms after the start of zoledronic acid was 46 months (range 24-66). Median number of cycles of bisphosphonate used was 44 monthly cycles (range 24-67). The commonest presentation was jaw pain, three patients had visible facial swelling. Two of the patients had non-healing sockets. Five patients were on concomitant chemotherapy (Thalidomide, cyclophosphamide, melphalan, interferon and steroids). Radiographs did show characteristic lytic lesions of multiple myeloma but as early inflammatory process is difficult to appreciate on x-rays only two patient films showed the typical healing response around the necrotic bone related to osteonecrosis of jaw. Biopsy of all patients was performed, tissues of non-healing sockets showed florid lymphoplasmacytic infiltrate, histopathology of the exposed bony patches revealed abundant purulent material and necrotic bone and laboratory findings of the patient having soft swelling in the anterior arch were presence of hyperplastic fibroepithelial growth. Orodental hygiene was poor in all patients. Only two patients had history of dental extraction. None of the patients had history of radiotherapy to head and neck. In all six patients zoledronic acid was stopped and was switched over to sodium clodronate. Debridement of non-healing sockets and exposed areas of bone were also carried out. Ptients were given long term antibiotics and were encouraged towards maintenance of good oral hygiene and regular use of antiseptic mouth washes. These measures brought substantive improvement to the quality of life of patients and relieved their symptoms to a certain extent however the lesions did not heal completely. After noticing these complications we discouraged the use of zoledronic acid outside the context of a clinical trial. Summary. Osteonecrosis of jaw following intravenous bisphosphonates has been noticed since 2003. Several case series have been published but as yet no proper guidelines for its prevention have been published. The incidence of jaw necrosis in patients with myeloma in most published series is about 4%. Majority of cases occur following zoledronic acid (80%) however pamidronate and even alendronate have been implicated. Dental procedures, poor orodental hygiene, radiotherapy and in some cases concomitant use of anti-angiotensinogenetic agents like thalidomide or corticosteroids are thought to be the commonest predisposing factors. In our short series of cases, we found a high incidence of (6/26) this complication in patients with multiple myeloma. This is worrying for the patients and treating physicians. Large case studies are warranted to delineate the predisposing factors and formulate management guidelines. Results of the U.K. MRC myeloma IX trial with regard to this complication in which patients with myeloma are randomized to receive zoledronic acid or intravenous pamidronate will be interesting. We suggest that physicians and dental community should liaise closely with each other in the identification and management of this dreaded complication. We suggest patients patients should be informed of the risk of osteonecrosis. All patients should be reviewed by the oral surgeons before the start of bisphosphonates and any dental infections removed.

**Table 1. Showing patients profil.**

<table>
<thead>
<tr>
<th>Pt.No</th>
<th>Age/sex</th>
<th>Date of diagnosis</th>
<th>Medications</th>
<th>No of infusions</th>
<th>Sign/symptoms</th>
</tr>
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<td>62/F</td>
<td>April 1999</td>
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<td>61</td>
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</tr>
<tr>
<td>2</td>
<td>56/F</td>
<td>April 1995</td>
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<td>67</td>
<td>Jaw pain</td>
</tr>
<tr>
<td>3</td>
<td>43/F</td>
<td>July 2003</td>
<td>Zoledronate</td>
<td>24</td>
<td>Non healing socket</td>
</tr>
<tr>
<td>4</td>
<td>68/F</td>
<td>Sep 1998</td>
<td>Thalidomide, steroids, Zoledronate</td>
<td>24</td>
<td>Jaw pain</td>
</tr>
<tr>
<td>5</td>
<td>68/F</td>
<td>April 1999</td>
<td>Cyclophosphamide, Zoledronate</td>
<td>58</td>
<td>Non healing socket</td>
</tr>
<tr>
<td>6</td>
<td>79/F</td>
<td>Sep 1999</td>
<td>Steroids, melphalan, Cyclophosphamide</td>
<td>28</td>
<td>Facial swelling</td>
</tr>
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**1416 CLINICO-BIOLOGICAL PROFILE AT FIRST PRESENTATION OF MULTIPLE MYELOMA PATIENTS. RETROSPECTIVE STUDY**

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**Backgrounds.** Despite all the clinical experience gained since now, the recognition of the disease in early stage continues to be unsatisfying. Aims. We analysed the clinico-biological profile of multiple myeloma (MM) patients at their first presentation in the clinic, in order to identify the stage and the factors which could increase the efficiency of the early diagnosis. Methods. We retrospectively analyzed a group of 125 patients with monoclonal gamapathy hospitalised in the Hematology Clinic Timisoara between January 1999 - May 2005. We studied the structure of the analysed group, the asymptomatic period in the disease evolution, the nature of the clinical manifestations, biological and paraclinical modifications, the structure of borrowed diagnostics, the hospi-
A median survival from ascites development was the same and very short regardless of ascites type and averaged 5.5 and 4.25 months, respectively. In MM two types of MM-related ascites are recognized. A low SAAG type with high cell counts is secondary to peritoneal involvement by MM with plasma cells proliferation or homing in the peritoneum. A high SAAG type with low cell counts is an example of mixed ascites with a myelomatous liver infiltration leading to portal hypertension as an additional pathogenetic factor. In both types an overlapping of failed lymphatic drainage may brings about a chylous ascites.

1418 INCIDENCE AND PRESENTATION OF OSTEONECROSIS OF THE JAWS IN MULTIPLE MYELOMA PATIENTS TREATED WITH BISPHOSPHONATES

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The administration of bisphosphonates is currently a mainstay in the management of hypercalcemia and bone absorption in a setting of malignancies, such as multiple myeloma or solid tumors. The main side effects of bisphosphonates are renal dysfunction, influenza-like syndrome and anemia. Osteonecrosis of the jaws (ONJ) is a recently reported complication of bisphosphonate use, especially intravenous formulations concerning. The unique properties of the oral cavity (bacterial flora, frequent injuries) combined with the antiangiogenic properties of bisphosphonates potentially contribute to the development of ischemic lesions in the jaws. The incidence of ONJ in MM patients treated with bisphosphonates is largely unknown as the diagnosis is usually made by oral and maxillofacial surgeons. We report six patients, 2 women and 4 men, (median age 65 years) with bisphosphonate-associated osteonecrosis of the jaws. MM therapy consisted of melphalan-prednisolone (4/6 patients), VAD-Caelyx or Thalidomide (one patient each). Four out of six affected patients were treated long-term (29 to 46 months) with either pamidronate or zoledronic acid for, respectively, 9 and 11 months only. Over the same five-year period, a total of 106 patients with MM were treated with bisphosphonates in our department. Therefore, the incidence of ONJ in our MM series was 6% (11/106 cases). Osteonecrosis typically presented with a painful exposed necrotic bone at the site of previous dento-extraction (5 patients) or surgery (1 patient). Biopsies of the jaw lesions showed no evidence of malignancy, while the cultures revealed normal oral flora. The lesions were not managed easily: most patients required surgical excision despite broad-spectrum antibiotic therapy and bisphosphonate discontinuation. One patient responded to hyperbaric oxygen therapy. The two patients who received bisphosphonates for less than a year showed a better response to therapy. In conclusion, osteonecrosis of the jaws is a serious complication of bisphosphonates which
requires alertness and prompt management. Rigorous oral hygiene and avoiding extensive dental procedures in patients who receive bisphosphonates could assist in preventing this complication.

Figure 1. ONJ in a bisphosphonate-treated MM patient.

1419
OSTEONECROSIS OF THE JAW IN MULTIPLE MYELOMA PATIENTS: MONOCENTRIC ANALYSIS

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Multiple myeloma (MM) is a common lymphoproliferative disorder. Modern therapies have remarkably improved the survival of affected patients. Thus, long term adverse events related to chemotherapy and/or ancillary treatments are observed with increasing frequency. Bisphosphonates (BP) are synthetic analogues of pyrophosphate. These compounds have been approved for treatment of cancer-related hypercalcemia and bone lytic involvement by MM and solid tumors. Zoledronic acid (ZA) is the most potent BP. It has antiangiogenic activity and inhibits osteoclastic differentiation and normal bone turnover. Recent studies have shown increased expression of C-X-C motif chemokine 12 (CXCL12) which is associated with osteoclasts activation. The objective of this monocentric analysis consists in the retrospective evaluation of the effects of treatment duration with ZA on the onset of ONJ in a cohort of 64 patients which were autotransplanted at our Institution. ONJ was evaluated by craniofacial complex CT and/or MRI followed by tissue biopsy for pathological and microbiological exams. Among the 64 analyzed patients, 5 (3 in the long lasting complete remission and 2 in very good partial remission) developed ONJ. Two of this also showed a massive necrosis of both maxillary sinuses. All of the patients referred pain and swelling, two of them also referred purulent discharge and necrotic jawbone exposure. ONJ was documented by biopsy in three of the five patients (3 men and 2 women). BP therapy was discontinued in all cases. Three patients underwent surgical curettage and all five were treated with antibiotic therapy. The outcome has been resolution of necrosis in three patients, persistence of bone exposure in one patient and oral analtral communication and cutaneous fistula in the other. Time to jaw osteonecrosis diagnosis since the beginning of BP treatment was three years in one patient, 4 years in two patients and 7 years in the last two. Osteonecrosis of the jaw in patients with MM can be associated with BP therapy. BF mechanism of action, that includes osteoclasts apoptosis and antiangiogenetic effect, is responsible for reduction of local blood flow and retard in bone repair. This leads to jaw bone damage. Duration of therapy with ZA is crucial in the development of this complication in affected patients.

1420
REMOVAL OF SERUM FREE LIGHT CHAINS BY HEMODIALYSIS IN PATIENTS WITH MULTIPLE MYELOMA

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Renal failure is a common complication of multiple myeloma (MM) and is associated with poor outcome. The cast nephropathy which results from the excess free light chain (FLC) production is the main cause of renal failure in MM. There is intense interest in whether rapid normalisation of serum FLC by plasma exchange (PE) and/or haemodialysis can improve renal outcomes. We have previously demonstrated that haemodialysis (HD) using high flux membranes removes a proportion of serum FLC in a non-MM, chronic HD population. However, in the MM setting, where serum FLC levels can be up to 10000 fold higher than normal, HD using standard high flux membranes was less effective. The purpose of this study was to demonstrate the ability of HD with a novel dialysis membrane to remove FLC in patients with MM. Five patients with MM and dialysis-dependent acute renal failure underwent dialysis using a Gambro Protein Permeable HCO 1100 Polyamide membrane (Gambro, Germany). Blood samples before and after each dialysis session were collected and samples of dialysate fluid were taken. Patients 1 to 5 had 5, 2, 6, 10 and 1 dialysis sessions for a mean time of 6, 4, 3, 4 and 6 hours respectively. FLC measurements were performed using the nephelometric immunoassay FREELITE™ (The Binding Site). To estimate the total amount of FLC removed, the following calculation was applied: mean FLC concentration in dialysate (mg/L) x dialysate volume (L). All 5 patients showed abnormal sFLC levels at the beginning of the study (3 with elevated serum free λ, patients 1 to 3), 2 with serum free kappa, patients 4 and 5). Dialysis using the Gambro HC1100 membrane was able to reduce sFLC levels in every session, for each patient. Although the amounts of FLC removed from the blood differ depending on the starting level, the mean percentage falls of light chain were 59.6%, 58.6% and 25.7% for the lambda patients and 45.9% and 61.8% for the kappa patients (Table 1). The means of the total amount of FLC removed per dialysis session were 35g, 31g, 8g, 20g and 4g for patients 1 to 5 respectively. We have conclusively demonstrated that HD can remove large quantities of FLC in the context of MM with the Gambro HC1100 dialysis membrane. Given that the total amount of FLC found in the dialysate exceeded the available FLC in the blood, these data suggest that FLC were also removed from the extravascular compartment. Serum FLC are known uremic toxins and contribute to worsening renal function and their rapid removal may be beneficial. The use of extended dialysis could further improve serum FLC removal in these patients.

Table 1. Table of FLC levels in serum and dialysis.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Dialysis</th>
<th>FLC type</th>
<th>Mean blood pre (mg/L)</th>
<th>Mean blood post (mg/L)</th>
<th>Mean% Removed</th>
<th>Total in dialysate fluid (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>λ</td>
<td>10626</td>
<td>4310</td>
<td>59.6</td>
<td>33</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>λ</td>
<td>9155</td>
<td>3760</td>
<td>58.6</td>
<td>31</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>λ</td>
<td>3362</td>
<td>2445</td>
<td>23.7</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>κ</td>
<td>2758</td>
<td>1489</td>
<td>45.9</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>κ</td>
<td>861</td>
<td>329</td>
<td>61.8</td>
<td>19.6</td>
</tr>
</tbody>
</table>

1421
QUANTITATIVE BONE ULTRASONOGRAPHY AT PHALANGES IN PATIENTS WITH PLASMA CELL DISCRASIAS AND ZOMETTA TREATMENT

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The current aims of bisphosphonates for metastatic bone disease are to prevent skeletal-related events (SREs), reduce bone pain and improve quality of life. Zoledronic acid (Zometa®) is the most potent tested bis-
phosphonate. Additional studies are needed to determine the optimal timing, schedule, and duration of treatment in myeloma patients. DBM Sonic Bone Profiler is the only ultrasound device that applies the method of signal analysis in transmission through phalanges. This technique of clinical investigation has proven to be particularly effective in post-menopausal osteoporosis for screening, diagnosis and therapies monitoring. To assess Bone Mineral Density (BMD) in patients with plasma cell menopausal osteoporosis for screening, diagnosis and therapies monitoring, our study includes 27 patients (pts) with TTP between 1986 and 2006. All patients had microangiopathic hemolytic anemia, thrombocytopenia, fever, fluctuating neurological symptoms and impaired renal function. Aims: To present the experience of the Department of Hematology, Clinical Center, Skopje, regarding this issue. Patients and Methods. our study includes 27 patients (pts) with TTP between 1986 and 2006. All patients had microangiopathic hemolytic anemia and thrombocytopenia. There were 14 women and 13 men; the median age was 36 years (range 20-71). It doesn't appear to be related to other diseases. Results. The mean period between the first symptoms and the diagnosis was 8.5 days (range 1-21). Neurological symptoms were present in 21 patient, bleeding in 19, fever in 6 and renal impairment in 12. Median hemoglobin was 68 g/L (range 40-95); median platelet count was 49x10^11/L (range 6-130); median reticulocytosis was 6% (range 1-25). Results of screening tests of coagulation showed elevation of EDF in 7 pts. Serum LDH was increased in all patients - median 1748 IU (range 618-4520). Treatment included: corticosteroids in all cases, exchange plasmapheresis (EP) in 12 pts, only plasma infusions in 13 pts, antiplatelet agents in 10 pts. Plasma exchange is currently not available in our country. In one patient with exacerbation during the first TTP episode treatment with Vincristine was introduced. There were 7 complete responders (5 on EP) and 14 deaths (3 on EP). Among the survivors 6 pts relapsed (2 pts had 2 relapses), 2 of them died during the first relapse; the median time from the onset of symptoms and treatment initiation lasted for 8.5 days (range 1-21), indicating poor disease recognition. The median time delay from diagnosis to EP was 5.5 days (1-11) suggesting relatively good EP availability. The median treatment duration in all patients was 15.5 days (range 1-40). The median number of EP cycles needed for the platelet stabilization was 4 (range 2-10). Conclusions. TTP is a severe disorder necessitating early recognition and diagnosis which would lead to treatment with EP on time. EP improves survival dramatically.

1422 WISKOTT-ALDRICH SYNDROME SHOULD MEDIUM PLAQUEPOSITORY VOLUME (MPV) MATTER?
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Background and Aims. Wiskott-Aldrich syndrome (WAS) is typically X-linked and is characterized by a clinical triad: eczema, thrombocytopenia with small platelets and immunodeficiency (predisposing to autoimmune phenomena, lymphoproliferative disease and neoplasia). However, there can be polymorphism on several levels - transmission autosomal dominant, transcription as or trans of the mutated gene that translate in different clinical evolution. We present a case of very early immunodeficiency, with a diagnostic being less obvious by the fact that the platelets always showed a normal MPV. Methods/Clinical Case: A caucasian male infant 1 month and 3 weeks old, without gestational or labour problems, is hospitalized with bloody diarrhea, petechiae on abdominal wall and mild squamous dermatitis. He presented Hb 8.5 g/dL, VGM 57.7%, reticulocytes 7.9%, leukocytes 13600/mm³ (atypical lymphocytes 10%), platelets 28300/mm³, LDH 1093. The marrow smear hasn't revealed maturation alterations on myeloid and erythroid series; lymphoid series -15% of small lymphocytes with high reason nucleus/cytoplasm; megacaryocytes diminished in number and in size with hypoproliferation of nuclei. The objective examination showed moderate pallor of skin and mucous membranes, peculiar facies (frontal lumps, short neck), dry skin with dermatitis (maculo-papular erithema) in the neck and retro auricular regions; liver 4 cm beyond costal grid, surpassing medium line; spleen 6 cm beyond costal grid. The diagnostic hypothesis were WAS, congenital or neonatal infection (TORCH) and auto-immune disease. Gradually a rise is seen in all liver enzymes; the thrombocytopenia Boats between 20 and 50,000/mm³ with MPV between 9 and 11FL and PDW of 80-100%. With further exams, autoimmune disease and TORCH infection are discarded; another remote hypothesis, Langerhans hysterosis was also discarded by the skin biopsy (compatible with atopic dermatitis). Clinical evolution between 3 and 7 months - 3 suppurated acute otitis, 2 gastro-enteritis and an interstitial pneumonia with severe hypoxemia (admittance in intensive care unit). The eczema has now a hemorrhagic component, with blood crusts, spreading from the face, neck, axila, dorsum and inguinal regions; hepato-splenomegaly has grown almost to iliac crest. He is medicated with co-trimoxazole and azithromycin (prophylaxis), folic acid and monthly palivizumab. It was done a complete immunologic study at 67 months old, that revealed: hypergammaglobulinaemia with hyperIgM (without deficit of IgG or IgA); lymphopenia T CDS; elevated expression of activation markers on T lymphocytes, however without proliferation after in vitro stimulation; NK lymphocytosis. Clearly having evidence of a primary or secondary T immunodeficiency, it is made a search for mutations ZAP-70 and WASP.

At 8 months old he is hospitalized by undetermined feverish syndrome and develops a very severe auto-immune hemolysis with shock that lead to his death. The confirmation arrives a mutation in exon 10 of WASP gene. Conclusions. In a case with an immunodeficiency this severe, briefly one has to think in bone marrow transplantation (the family was already being HLA typed). WASP protein seems to be involved in mechanisms of signal transduction between surface receptors and cytoskeleton, provoking defects of chemotaxis, fagocytosis and presentation of antigens to T cells with inappropriate response. In platelets, there are diminished surface glycoproteins and adhesion defects. In literature, we find more and more heterogeneous cases, whether in genetic/phenotypic expression or clinical expression (as platelets with normal MPV), that can help achieving a better understanding upon mechanisms of WAS.

1423 THROMBOTIC THROMBOCYTOPENIC PURPURA: REPORT OF 27 CASES
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Clinical Center, SKOPJE, Macedonia

Thrombotic thrombocytopenic purpura (TTP) is a severe multisystemic disorder characterized by microangiopathic hemolytic anemia, thrombocytopenia, fever, fluctuating neurological symptoms and impaired renal function. Aims: To present the experience of the Department of Hematology, Clinical Center, Skopje, regarding this issue. Patients and Methods. Our study includes 27 patients (pts) with TTP between 1986 and 2006. All patients had microangiopathic hemolytic anemia and thrombocytopenia. There were 14 women and 13 men; the median age was 36 years (range 20-71). It doesn’t appear to be related to other diseases. Results. The mean period between the first symptoms and the diagnosis was 8.5 days (range 1-21). Neurological symptoms were present in 21 patient, bleeding in 19, fever in 6 and renal impairment in 12. Median hemoglobin was 68 g/L (range 40-95); median platelet count was 49x10^11/L (range 6-130); median reticulocytosis was 6% (range 1-25). Results of screening tests of coagulation showed elevation of EDF in 7 pts. Serum LDH was increased in all patients - median 1748 IU (range 618-4520). Treatment included: corticosteroids in all cases, exchange plasmapheresis (EP) in 12 pts, only plasma infusions in 13 pts, antiplatelet agents in 10 pts. Plasma exchange is currently not available in our country. In one patient with exacerbation during the first TTP episode treatment with Vincristine was introduced. There were 7 complete responders (5 on EP) and 14 deaths (3 on EP). Among the survivors 6 pts relapsed (2 pts had 2 relapses), 2 of them died during the first relapse. The median time to relapse from the onset of symptoms and treatment initiation lasted for 8.5 days (range 1-21), indicating poor disease recognition. The median time delay from diagnosis to EP was 5.5 days (1-11) suggesting relatively good EP availability. The median treatment duration in all patients was 15.5 days (range 1-40). The median number of EP cycles needed for the platelet stabilization was 4 (range 2-10). Conclusion. TTP is a severe disorder necessitating early recognition and diagnosis which would lead to treatment with EP on time. EP improves survival dramatically.

1424 A CORRELATION STUDY BETWEEN SERUM POTASSIUM LEVEL AND AETIOLOGY OF THROMBOCYTOPENIA.
L. Ong
Ulster Hospital Dundonald, BELFAST, United Kingdom
A good response to corticosteroids which is used as first line treatment of immune thrombocytopenic purpura (ITP). Post-splenectomy late response in platelet aggregation can be both indirect and direct. AVP synthetic analog desglycilamide-arginine vasopressin (DGAVP) as inductor of platelet aggregation in man or rat PRP but its effect is more intense. The aim of this study was to compare effect of AVP and it's analog DGAVP as natural peptide AVP and DGAVP as inductor of platelet aggregation. Effect of AVP and DGAVP as inductor of platelet aggregation was studied on platelet-rich blood plasma (PRP) in the children without bleeding disorders or in children who had got platelet aggregation disorders with ADF. Thus we conclude that AVP analog DGAVP as natural vasopressin induces platelet aggregation in man or rat PRP but it's effect is more intensive. Besides our results demonstrate that as DGAVP as AVP effect on platelet aggregation can be both indirect and direct.

**EFFECT OF VASOPRESSIN AND IT'S ANALOG DGAVP ON PLATELET AGGREGATION**

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1Moscow State University, MOSCOW, Russian Federation; 2Res. Inst. of pediat-ric Hematology, MOSCOW, Russian Federation

It is well known that neurophysiological peptide vasopressin (AVP) causes blood coagulation due to increased secretion of tissue thrombo-plastin and factor VIII:C into blood. Besides AVP exerts significant influence on platelet aggregation through interaction with specific receptors. AVP synthetic analog desglycilamide-arginine vasopressin (DGAVP) lacks peripheral hormonal activity and evokes blood procoagulant activity too. The aim of this study was to compare effect of AVP and it's analog DGAVP as inductor of man or rat platelet aggregation. Effect of AVP and DGAVP as inductor of platelet aggregation was studied on platelet-rich blood plasma (PRP) in the children without bleeding disorders or in children who had got platelet aggregation disorders with ADF. Thus we conclude that AVP analog DGAVP as natural vasopressin induces platelet aggregation in man or rat PRP but it’s effect is more intensive. Besides our results demonstrate that as DGAVP as AVP effect on platelet aggregation can be both indirect and direct.

**TREATMENT OF IMMUNE THROMBOCYTOPENIC PURPURA. POSTSPLENECTOMY LATE RESPONSE**

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1UMF Victor Babes, TIMISOARA, Romania; 2County Hospital, TIMISOARA, Romania

Backgrounds. Immune thrombocytopenic purpura (ITP) is a chronic disease with a good response to corticosteroids which is used as first line therapy. Some times after steroid therapy patients relaps and in this case spleenectomy in the second line of therapy. Aims: We try to evaluate the therapeutic results in a group of patients with ITP and the duration of their remission after splenectomy. Methods. From may 1990 - may 2000 we hospitalised and treated in the Hematology Clinic 125 patients with ITP. Median age was 48 years with a distribution on sexes: 46 males and 89 females. Our patients 91% presented gastrointestinal bleeds, 52% had sclerodermatous bleedings and 9% had bleedings in the central nervous system. The most of the patients 91% were treated with corticosteroids, 12% received steroids and immunoglobulins. 35% (47 patients) had a splenectomy because they relapsed after steroids or they needed very high doses of corticosteroids for a safe number of trombocytes. From those 47 patients with splenectomy 19 were males and 28 were females with a median age at the time of splenectomy of 88 years. The medium time from diagnosis to splenec-tomy was 3,5 years (0,6-96 months) The response to splenectomy was defined as followes: complete response (CR) a number of trombocytes higher than 150.000/mms for more than 4 weeks, partial response (PR) trombocytes between 50.000-150.000/mms lasting more than 4 weeks and relapse a number of trombocytes under 50.000/mms. Results. The medium follow-up time was 7 years (2-10 years). The overall response was 79% with 58% of CR and 21% of PR. From 47 patients with splenec-tomy 15 patients relapsed and 5 of this 15 were in CR after steroid therapy following splenectomy. The long term follow-up in CR and PR proves a good, stable and durable response in time for more than 7 years. Post splenectomy complications in the study group were not signifi-cant. Conclusions. Our study proves that patients with chronic immune thrombocytopenic purpura who failed corticoetherapy get a safe and durable response in time after splenectomy.

**INADEQUATE RESPONSE TO RITUXIMAB IN PATIENTS WITH CHRONIC REFRACTORY IMMUNE THROMBOCYTOPENIA (ITP)**

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Beaumont Hospital, DUBLIN, Ireland

**Table 1. Rituximab in Chronic Refractory ITP.**

<table>
<thead>
<tr>
<th>Patients</th>
<th>Age</th>
<th>Sex</th>
<th>Co-morbidity</th>
<th>Treatment Pre</th>
<th>Platelet Count</th>
<th>Platelet Count</th>
<th>Treatment After</th>
<th>Response</th>
<th>Time</th>
<th>Maximum Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>35 yr/F</td>
<td>Nil</td>
<td>Ig + Steroids</td>
<td>36</td>
<td>40</td>
<td>Anti-D</td>
<td></td>
<td>Minimal</td>
<td>4 wk</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>50 yr/M</td>
<td>NIDDM</td>
<td>Hypercholesterolemia</td>
<td>Ig + Steroids</td>
<td>69</td>
<td>70</td>
<td>Nil</td>
<td></td>
<td>Not Applicable</td>
<td>(NA)</td>
</tr>
<tr>
<td>3</td>
<td>68 yr/F</td>
<td>Osteoarthritis</td>
<td>Ig + Anti-D Danazol</td>
<td>Ig + Steroids</td>
<td>34</td>
<td>20</td>
<td>Ig</td>
<td></td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>80 yr/F</td>
<td>-</td>
<td>Ig + Danazol</td>
<td></td>
<td>29</td>
<td>28</td>
<td>Splenectomy</td>
<td></td>
<td>NA</td>
<td></td>
</tr>
</tbody>
</table>

Methods. We have used 4 cycles of Rituximab (375 mg/m2) adminis-tered at weekly intervals to 4 patients (3 females, 1 male; mean age 58 years) with chronic refractory ITP. All 4 patients had previous treatment with Immunoglobulins. Three patients had had prior treatment with Prednisolone; one did not receive Prednisolone because he was a Diabetic. One out the four patients had also received prior treatment with Anti D and Danazol (along with Immunoglobulins and steroids).
Response Criteria: A Complete Response (CR) was defined as a rise in platelet count >100 × 10⁹/L, a Partial Response (PR) as a rise in platelet count 50-100 × 10⁹/L and a minor response (MR) as a rise in platelet count <50 × 10⁹/L. No Response (NR) was defined as no increase in platelet count.

Results. Of the 4 patients 1 had a Minor Response and 3 had No Response. However Rituximab was well tolerated in all 4 cases with no major side-effects. Conclusions. Our results suggest that Rituximab hardly made any impact on the platelet count of these 4 patients with chronic refractory ITP. Previous studies of Rituximab in ITP has shown an enhanced thrombopoiesis with obesity-related metabolic and vascular diseases, the role of platelet abnormalities are frequently found in these patients and range from platelet hypofunction, acquired storage pool disease and/or platelet membrane defects, to abnormalities suggesting increased platelet reactivity, increased plasma β-thromboglobulin levels or shortened platelet survival. In the present study, we aimed to investigate platelet function abnormalities using both optical platelet aggregometry and whole blood platelet aggregometry and evaluate the effects of various therapeutic regimes on these abnormalities, in patients with MPD. 45 patients with newly diagnosed chronic myeloproliferative disorders (26 CML, 11 PCV, 8 ET) were enrolled. Median age was 54; there were 23 females and 22 males. At the study entry, whole blood count, PT, aPTT, fibrinogen, platelet aggregation studies by luminescence method in whole blood and by optical method in PRP, ristocetin cofactor activity were performed. The agonists used were; ADP, Arachidonic acid (AA), Ristocetin and Collagen. Platelets were considered to be hyperactive if at least one result (aggregation or ATP release with one agonist) was above the reference range, and hyporeactive if at least one result (aggregation or ATP release with one agonist) was below the reference range. Mixed hypo- and hyperactive platelets were considered present when at least one result (aggregation or ATP release) was above and below the reference range, respectively. Repeat platelet function studies were performed in 20 patients, following specific therapy regimes. By luminescence method; before therapy 15/45 patients had platelet hyperfunction, 17/45 patients had coexistence of hyper- and hypofunction and 12/45 patients had platelet hypofunction. 1/45 patient had a normal result. After therapy 13/20 patients had platelet hypofunction, 2/20 patients had platelet hyperfunction, 2/20 patients had coexistence of hyper- and hypofunction while 3/20 patients had normal results. By optical method; before therapy 18/45 patients had platelet hyperfunction, 9/45 patients had platelet hyper- and hypofunction, 7/45 patients had platelet hyperfunction whilst 11 had normal results. After therapy 15/20 patients had coexistence of hyper- and hypofunction, 4/20 patients had platelet hyperfunction, 1/20 patients had platelet hypofunction while none of the patients had normal results. We conclude that; 1. Different platelet function defect is observed in most of patients with MPD. 2. Patients with CML have platelet hypogagregability while patients with PCV and ET have platelet hyperaggregability. 3. Our observations highlight the need to use WBPA to select patients for antiplatelet therapy in MPD. 4. Luminescence method appears to be more sensitive than optical method to evaluate platelet functions.

1428 EFFECTS OF VARIOUS THERAPEUTIC REGIMENS ON PLATELET FUNCTIONS IN PATIENTS WITH MYELOPROLIFERATIVE DISORDERS M. Akay, E. Akin, Z. Gulbas Eskisehir Osmangazi University Hospital, ESKESEHR, Turkey

Bleeding and thrombosis are common causes of morbidity and mortality in patients with myeloproliferative disorders (MPD). Qualitative platelet abnormalities are frequently found in these patients and range from platelet hypofunction, acquired storage pool disease and/or platelet membrane defects, to abnormalities suggesting increased platelet reactivity, increased plasma β-thromboglobulin levels or shortened platelet survival. In the present study, we aimed to investigate platelet function abnormalities using both optical platelet aggregometry and whole blood platelet aggregometry and evaluate the effects of various therapeutic regimes on these abnormalities, in patients with MPD. 45 patients with newly diagnosed chronic myeloproliferative disorders (26 CML, 11 PCV, 8 ET) were enrolled. Median age was 54; there were 23 females and 22 males. At the study entry, whole blood count, PT, aPTT, fibrinogen, platelet aggregation studies by luminescence method in whole blood and by optical method in PRP, ristocetin cofactor activity were performed. The agonists used were; ADP, Arachidonic acid (AA), Ristocetin and Collagen. Platelets were considered to be hyperactive if at least one result (aggregation or ATP release with one agonist) was above the reference range, and hyporeactive if at least one result (aggregation or ATP release with one agonist) was below the reference range. Mixed hypo- and hyperactive platelets were considered present when at least one result (aggregation or ATP release) was above and below the reference range, respectively. Repeat platelet function studies were performed in 20 patients, following specific therapy regimes. By luminescence method; before therapy 15/45 patients had platelet hyperfunction, 17/45 patients had coexistence of hyper- and hypofunction and 12/45 patients had platelet hypofunction. 1/45 patient had a normal result. After therapy 13/20 patients had platelet hypofunction, 2/20 patients had platelet hyperfunction, 2/20 patients had coexistence of hyper- and hypofunction while 3/20 patients had normal results. By optical method; before therapy 18/45 patients had platelet hyperfunction, 9/45 patients had platelet hyper- and hypofunction, 7/45 patients had platelet hyperfunction whilst 11 had normal results. After therapy 15/20 patients had coexistence of hyper- and hypofunction, 4/20 patients had platelet hyperfunction, 1/20 patients had platelet hypofunction while none of the patients had normal results. We conclude that; 1. Different platelet function defect is observed in most of patients with MPD. 2. Patients with CML have platelet hypogagregability while patients with PCV and ET have platelet hyperaggregability. 3. Our observations highlight the need to use WBPA to select patients for antiplatelet therapy in MPD. 4. Luminescence method appears to be more sensitive than optical method to evaluate platelet functions.

1429 ADIPONECTIN ADDED INTO THE PLASMA OF HEALTHY PROBANDS DOES NOT AFFECT PLATELET AGGREGABILITY J. Prosikova, D. Stejskal Sternberk Hospital, STERNBERK, Czech Republic

Background. Adiponectin exhibits important antidiabetic and antiatherogenic effects. Although hypoadiponectinemia is associated with obesity-related metabolic and vascular diseases, the role of adiponectin in thrombosis remains elusive. Recent paper informed that adiponectin deficiency in adiponectin knockout male mice leads to enhanced thrombus formation and platelet aggregation. Aims. Evaluate the effects of added adiponectin on the plasma in platelet aggregability. Methods. 6 healthy nonobese healthy probands were tested. In all of them platelet aggregability and adiponectin values were measured. Human adiponectin (Biovendor; Czech Republic) was added to PRP in different concentrations (100; 75; 50 and 25 ng/l). Than PRP was 5 min incubated and was evaluated induced platelet aggregation using CPG (Analytical Control Systems) at 3 μmol/l as the final concentration of CPG added to PRP with an Apact II platelet aggregometer (Labitec GmbH). Induced aggregation extent was defined by the slope of aggregation curve. Results. Adiponectin values had normal distribution in tested group (13,7-15,8 ng/l). Neither of tested probands had significant difference of the slope CPG values, even if 100 ng/l adiponectin concentration was added. Conclusions. The present study did not verify hypothesis about the inverse correlation between human hyperadiponectinemia as an antithrombotic factor. Adiponectin concentration about 10 ng/l have similar antithrombotic effect as in vitro action as values upper 100 ng/l.

1430 PRESENTATION OF NEW METHOD FOR ASA RESISTANCE DETECTION J. Prosikova, D. Stejskal Sternberk Hospital, STERNBERK, Czech Republic

Backgrounds. Aspirin resistance seems to be an important prognostic factor in patients with coronary artery disease, but there is limited data on its correlation to clinical outcomes. Various methods for both in vivo and in vitro platelet function exist. In late 1990s, a novel in vitro inducer of platelet aggregation - cationic propyl gallate (CPG) was introduced into clinical practice, announced as an unprecedented, highly sensitive and specific method for assessment of aspirin resistance. In classic aggregometry problem with patients compliance remain unresolved. Recently there were presented information about chance for ASA resistance testing by virtue of in vitro aggregation test with ASA addition. Aims. Evaluate platelet ASA resistance with platelet CPG aggregation after ASA addition.

Methods. 20 healthy individuals and 20 patients with metabolic syndrome were evaluated. No individuals were ASA treated. In all of probands was performed platelet aggregation (Multiplate) after CPG induction. In part of whole blood was supplement solution of ASA (Aspisol, Bayer) and was perform aggregometry, over again. Results. Healthy probands have higher difference between AUC before and after the ASA pretreatment. (p<0,01, Kruskal Wallis) than probands with metabolic syndrome. CPG have higher difference before collagen (p< 0,05). AUC of aggregometry line in all of healthy probands had significant reaction after Aspisol addition. On the contrary, AUC of patients with metabolic syndrome reacted different. Conclusion: authors presented frequent ASA resistance existence in individuals with metabolic syndrome against healthy. At the same time presented new in vitro method for ASA resistance detection which eliminate patient non compliance errors.
1431

ASPIRIN STRENGTHENS ANTITHROMBOTIC EFFECT OF HEPARIN-LIKE ANTICOAGULANT FROM PLANT

Y.U. Lyapin, Y.U. Obergan
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Background. Effective antithrombotic agents possessing antiplatelet activity, are preparations such as aspirin, dipiridamol and others. Low molecular weight heparin also shows antithrombotic activity. Antithrombotic effect of plant heparin-like anticoagulant from roots of a peony at chronic intranasal application has been shown. Aims. The purpose of the present work consist in studying antithrombotic effects of antiplatelet aspirin at the purpose of the present work consist in studying antithrombotic effects of antithrombotic heparin (PH) from Paeonia suffruticosa with antiplatelet aspirin at the purpose of the present work.

Methods. In the work methods electrophoretic, chromatographic and spectral analysis were used. The formation of blood clots was carried out on method Wessler with our updating - in isolated by metal clips a fragment vein (jugular). PH (1mg/mL) and aspirin (1 mg/mL) mixed in the ratio 1 : 1 (w/w). This mix was administrated peroral to animals (albino rats) in daily volume 0.5 ml/kg of body weight within 7 days before formation of blood clots. Antithrombotic effect was estimated on frequency of cases of thrombus formation and on weight of blood clots after 1 hour after thrombus formation. Results. At formation of blood clots of animals on a background of action PH + aspirin thrombus either were not formed (did not come to light) or were small (in case of their formation). So, quantity of cases of thrombus formation in experiment on a background administrated PH + aspirin made 8 per cent, on a background administrated one PH made 55 per cent from the control (administration of 0.85% solution of NaCl). The average weight of the formed blood clots in experiment with PH + aspirin made only 4-6 per cent and with PH - 15-16 per cent from the control (100 per cent). Furthermore it has been marked any collateral negative influences PH + aspirin and on PH - (for example, haemorrhagic action). Conclusion. Administration aspirin together with PH has shown higher antithrombotic effect against one PH. The difference in antithrombotic effectiveness between PH + aspirin and the PH was 10 -11 per cent. So, we have established, that aspirin strengthens antithrombotic effect of low-molecular plant heparin.

1432

SUCCESSFULL TREATMENT OF HEPARIN INDUCED THROMBOCYTOPENIA TYPE II AND THROMBOSIS WITH FONDAPARINUX IN A DIALYSIS PATIENT

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Although rare heparin induced thrombocytopenia (HIT) type II is one of the most feared complications of heparin therapy, Unfractionated heparin is the standard anticoagulation used in hemodialysis. Dialysis patients who are continually exposed to heparin are at risk for HIT. We report a 75-year-old male patient with acute-on-chronic renal failure who subsequently developed HIT while on hemodialysis. The patient admitted to our emergency department with dyspnea, tachypnea and bilateral low chest pain. Hematologic examination and laboratory assessment revealed acute lung edema, uric acidosis and acute-on-chronic renal failure requiring urgent renal replacement therapy. His history revealed hypertension for 15 years and diabetes for 1 year. A double lumen hemodialysis catheter was inserted into the left femoral vein and regular hemodialysis therapy with unfractionated heparin as anticoagulant was started. 10 days after starting hemodialysis the platelet count dropped from 504000/mm² to 470000/mm² and there were pain and swelling of his left leg. Doppler ultrasound examination showed left femoral vein thrombus formation. Thereafter enoxaparin therapy was started for deep vein thrombosis. Five days later when platelets were found to be 22000/mm² the patient was consulted with one of our hematology team members. As the patient had no other explanation for thrombocytopenia HIT type II was strongly suspected. Anti-heparin platelet factor-4 (Diagnostica Stago, France) complexes were positive (OD 2,375). Both functional assays, heparin-induced platelet aggregation test (HIPA) and C14-serotonin release assay were positive. Plasminogen activator type heparin and unfractionated heparin during hemodialysis sessions were stopped. Fondaparinux 2.5 mg daily was started. We could not monitor the anti-Xa activity because technical problems. During fondaparinux treatment platelet count increased to 193000/mm² and repeated Doppler ultrasound showed recanalization of left femoral vein thrombus. When the platelet count reached 100000/mm² oral anticoagulation with warfarin was initiated. The dose of warfarin was adjusted to maintain a target INR of 2.5. When INR was therapeutic for two consecutive days fondaparinux was stopped. During follow-up no new thromboembolic attack was observed. We present a patient with HIT type II and femoral vein thrombosis while on dialysis who was successfully treated with fondaparinux. In this case HIT type II and catheter-induced vessel wall damage were two independent risk factors for venous thrombosis. As HIT type II is a life threatening complication of heparin therapy all physicians using heparin anticoagulation should be aware of it. For all patients receiving unfractionated heparin alternate day platelet counts should be performed from days 4 to 14. Concern about fondaparinux is too small to be recognized by the majority heparin-reactive antibodies it could be a reasonable alternative anticoagulant for symptomatic HIT type II patients where licensed drugs like lepirudin and danaparoid are not available.

1433

INCREASED RISK OF DEVELOPMENT OF HEPARIN INDUCED THROMBOCYTOPENIA (HIT) IN ICU CRITICALLY ILL PATIENTS WITH VENOUS OR ARTERIAL LINES IN PLACE AND/OR NEED OF RENAL REPLACEMENT THERAPY WITH CONTINUOUS VENOUS-VENOUS HEMODIAFILTRATION (CVVHF)

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To investigate whether the use of heparin as anticoagulant in either central venous, arterial or CVVHDF catheters ,as well as Low Molecular Weight Heparins(LMWH) increases the risk of HIT. The cases with confirmed HIT were included in the study. Thirty (30) patients (17 men and 13 women) aged 31-87 years ,admitted in the ICU for various reasons during 2004-2005 were included in the study. At admission APACHE II score was <20 in 14 patients and > 20 in the remaining 16 patients. All patients had arterial or central venous catheters flushed with small quantities of unfractionated heparin.In 14 patients (group A) LMWH was administered in every day basis as prophylaxis from deep vein thrombosis(enoxaparine 2000-4000 IU/day), while 6 patients (groupB) underwent CVVHDF using unfractionated heparin as anticoagulant. Platelet measurements were performed in all patients at day 1st, 7th and 15th with hematology analyzer ADVIA120.Antibodies against the complex heparin-PF4 with elisa (As erachrom EPIA, Stago) were tested in all patients at day 7 and 16 after catheter insertion.

<table>
<thead>
<tr>
<th>Catheters +LMWH</th>
<th>Catheters + CVVHDF</th>
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<tr>
<td>Group A</td>
<td>Group B</td>
</tr>
<tr>
<td>n=24</td>
<td>n=6</td>
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<tr>
<td>1st day</td>
<td>7th day</td>
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<td>Thrombopenia</td>
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From all patients studied (30), at day 7,12 patients from group A and 2 patients from group B were positive for HIT-IgG antibodies. In addition, at day 15 ,other 5 patients (4 from groupA and 1 from group B ) were positive for HIT-IgG detection.Overall, 17 patients (56,6%) developed HIT-IgG antibodies (seroconversion).Thrombocytopenia (HIT) was detected in 2 patients (6,6%). All patients with HIT-IgG antibody underwent vascular imaging (triplex) in order to exclude subclinical thrombosis.No correlation was found between severity score (APACHE II) and presence of HIT-IgG antibodies. According the results of this study, combination of heparinized catheters or use of LMWH seems to increase the incidence of HIT(6,6%) as well as the development of HIT-IgG antibodies (seroconversion) in about 56,6% of patients. On the other hand, combined use of heparinized catheters and CVVHDF filters seems to increase the presence of HIT-IgG antibodies (seroconversion) in high percentage of patients (50%). The management of ICU patients with HIT includes:

- Discontinuation of LMWH
- Flushing catheters with normal saline
- Use of filters without need of heparin.
Seminal Factor VII and VIII: The Ultimate Evidence on the Presence of the Tissue Factor Dependent Pathway in Human Semen

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Backgrounds. Human semen spontaneously coagulates into a semisolid mass and then wholly liquefies in a process that may have some similarity to that of blood. Besides other active components of the haemostatic system, semen contains a significant amount of functional tissue factor (TF). Aim: To investigate the presence of Factor (F) VII and FVIIa in human semen. Materials and Methods. Using a PT/APTT one stage factor assay and an Imubind™ FVIIa ELISA-assay, FVII and FVIIa levels were assessed in 97 semen specimens obtained from sub-fertile, normally fertile, fertile sperm donors and vasectomized subjects. Results. FVII and FVIIa were quantifiable in human semen. The mean FVII levels were 4.4 IU/dL and FVIIa were 12 ng/mL. Despite the observed variations in seminal FVIIa levels we found no significant differences in FVIIa levels among the studied groups. Seminal FVIIa levels showed a significant positive association with semen liquefaction time, sperm motility and semen volume. The anti-sperm antibodies and sperm agglutination groups also showed raised FVIIa levels. We found no relationship between FVIIa levels and total sperm concentration (density), sperm counts per ml, sperm progression and days of abstinence. Conclusion. The present finding reinforces the concept of an active clotting system in human semen, not least the presence of the TF-dependent pathway.

No Effect B-Vitamin Supplementation on Markers of Inflammation in Patients with Venous Thromboembolism

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Backgrounds. Mild hyperhomocysteinemia is associated with an increased risk of venous thromboembolism (VTE) and other cardiovascular diseases. Recent studies have suggested a role of inflammatory markers in the etiology of VTE and elevated homocysteine levels may contribute to low-grade inflammation. Vitamin supplementation with folic acid and B-vitamins was previously shown to decrease homocysteine and homocysteine reduction by B-vitamin supplementation had no effect on these markers even in patients above the highest tertile. However, the levels of hsCRP and IL-8 did not change both in the vitamin and placebo treated patients. Besides, treatment with vitamins had no effect on these markers even in patients above the highest tertile of homocysteine. Conclusions. In patients with VTE, higher homocysteine levels were not associated with increased levels of inflammation markers and homocysteine reduction by B-vitamin supplementation had no effect on these markers.

Primary Hemophagocytic Syndrome Case Report

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An 11-year-old white girl with good features presented with a history of fever, somnolences, jaundice and petechia. She had a past history of jaundice and elevation of transaminases five months ago which resolved without therapy. There was hepatomegaly and splenomegaly 3 and 4 cm respectively. Laboratory findings were: Hb: 8.4 g/dL, WBC: 1600/mm³, PLT: 1800/mm³, ALT: 290 U/L, AST: 129 U/L, Tbilirubin: 12.5 mg/dL, D.Bil: 4.5 mg/dL, PT: 17.4 sec, PTT: 42 sec, INR: 1.5, fibrinogen: 90 mg/dL, serum ammonia: 150 mg/dL. Cerebrospinal fluid examination was normal. Clinical presentation was suggesting hemophagocytic lymphohistiocytosis (HLH) but bone marrow aspiration did not confirm the diagnosis. Marrow biopsy revealed hemophagocytosis. Hepatitis B, Hepatitis A, CMV, EBV, HSV, parvovirus serology did not show acute infection. Hepatit A IgG was positive, HCV RNA was negative. HLH-94 protocol was started and fever, hepatosplenomegaly and somnolences subsided but only partial hematological remission could be achieved (Hb: 6.1 g/dL, WBC: 4900/mm³, PLT: 47000/mm³). ATG 10 mg/kg/day for three consecutive days were also administered and complete hematological and biochemical remission was achieved. Parents were cousins but there was no history of similar disease. Genetic study could not be performed to confirm primary HLH. Following three uneventful years the patient was referred to our center again with a 5 month history of bilateral abducens paralysis and ataxia. Cranial MRI showed increased T2 signal in cerebellar, supratentorial areas, right occipital deep white matter, bilateral thalamus and centrum semiovale and diffuse cerebellar and mild cortical atrophy. HLH-94 protocol was again started but progression was seen with head tremor, generalized clonic convolution, fever (59°C axillary) and cytopenias. Hb: 10.6 g/dL, WBC: 2150/mm³, ANC: 678/mm³, platelet: 11600/mm³. Genetic study showed homozygous perforin mutation that leads to aminoadipic acid exchange from (Val50Thr). We are planning allogeneic stem cell transplantation from siblings if they do not show the same homozygous perforin mutation because clinical presentation might be late as seen in the patient. HLH must be remembered in the differential diagnosis of fever, cytopenias, splenomegaly, hepatic failure and neurological symptoms. Delay in diagnosis may impair outcome. Allogeneic stem cell transplantation is the only curative therapy in primary disease.

Herpes Group Virus Infections and Granulocytotoxic Antibodies in Children with Neutropenias

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Relation between the duration of granulocytotoxic antibodies (GCTA) circulation and the presence of herpetic infection has been studied in children with immune neutropenias. Group 1 consisted of 15 herpes group virus-infected children with immune neutropenia aged 4 to 24 months; virus infections included cytomegalovirus (n = 10), Epstein-Barr virus (n = 1), herpes simplex virus 1 or 2 (n = 2) and mixed infection (n = 2). GCTA circulation lasted for 0.5 to 3 months (1.67 ± 1.25). GCTA titers ranged from 1.2 to 1.64. No correlation between GCTA titers and duration of GCTA circulation has been revealed. Group 2 consisted of 18 children aged 6 to 12 months with immune neutropenia and no markers of herpetic and clinical group viremia. GCTA circulation lasted for 6.96 ± 0.33 months. GCTA titers ranged from 1.2 to 1.156 and, similarly to those in Group 1, caused no effect on the duration of GCTA circulation. Thus, statistically significant difference in duration of GCTA circulation (p < 0.001) between the studied groups has been found; this result indicates the presence of a pathogenic role of herpes group viruses in the immune conflict in children with immune neutropenias.

The Value and Limitations of WBC Differential Flags Provided by the Automated Hematology Analyser Sysmex XT 2000i

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Backgrounds. Sysmex XT 2000i is a fully automated hematology
analysed with a throughput of 80 samples per hour. The analyser can provide 88 parameters including 76 parameters and complete blood count in addition to neutrophilic differential using flow cytometry by semiconductor laser enabling a sophisticated analyses based on RNA/DNA content, cell size and inner cell complexity. The analyser detects the presence of abnormal or immature WBC, providing a list of suspect messages generated from abnormal cell locations on WBC/DIFF scattergram. Aims: The present study evaluates the diagnostic performance of Sysmex XT 2000i in detection of abnormal or immature WBC in comparison with manual microscopy review and also the value of flags in leucocyte count. Methods: In this study we included 100 samples. All venous blood specimens were collected from 100 patients admitted in Hematology Department between July 2006 and February 2006 and diagnosed as acute leukemia (44 cases), CLL (15), malignant lymphoma (15), chronic myeloproliferative disease (9), MDS (8), anemia (5), trombocytopenia (3), infectious mononucleosis (2), HCL (1), TTP (1), MM (1). Clinical sensitivity and specificity of suspect flags of XT were assessed by comparison with microscopy differential counts. Sysmex XT 2000i generated messages of blasts in 95% of cases confirmed in 61% with optic microscopy. In the group of 44 cases with acute leukemia, XT flagged blasts in 41 cases (93%) comparable with optic microscopy which detects blasts in 43 cases (98%). In MDS cases (8), 5 samples (62%) were flagged on XT and optic microscopy confirmed presence of blasts in 8 all cases. 15 samples of CLL were false positive for blasts on XT, in all cases we found mature lymphocytes. In samples flagged with blasts on XT need a manual microscopy review. Sysmex XT 2000i shows high sensitivity (95%) and lower specificity (50%) in detections of blasts, provide reliable results and a WBC differential comparable with optic microscopy.

**1440**

**ORAL VORICONAZOLE AS SECONDARY PROPHYLAXIS DURING ALLOGENIC STEM CELL TRANSPLANTATION IN A PATIENT WITH PREVIOUS SEVERE INVASIVE FUSARIOSE SP INFECTION**


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Invasive fungal infections (IFI) represent one of the most challenging complications in patients submitted to stem cell transplantation (SCT). Invasive fusariosis have a high mortality rate in immunocompromised patients. Retrospective analysis of 69 brazilians patients (Nucci et al, CID 2004) showed a median survival of only 13 days. None of this patients, although received a newer antifungal drug, such as Voriconazole. Some studies have showed the safety and efficacy of Voriconazole as a secondary prophylaxis during SCT in patients with previous Aspergillus sp infection. However, to our knowledge, the use of Voriconazole as a secondary prophylaxis in SCT in patients with previous invasive Fusarium sp infection has not been reported yet. Case Report. A 49 years old man, with the appearance of skin lesions and important myalgia a clinical suspicion of fusariosis was made and oral Voriconazol was started. Fusarium sp blood culture sample confirmed the diagnosis of invasive fusariosis. He achieved complete remission after salvage chemotherapy and used oral Voriconazole for 2 months. The latest pulmonary CT scans showed a very small residual lesion in the lower lobe of the right lung. With no other sibling identical donors, we submitted him to a second SCT with a different donor. During the whole conditioning period (Bussulfan 16 mg/kg and Fludarabine 120 mg/m²) and until day + 6 he received 400 mg of oral Voriconazole. Liver and renal test were undertaken daily, and Cyclosporine level were measured twice a week. With a slight increase in bilirubin on day + 6 (6,48 mg/dL) Voriconazole was stopped for two days and reintroduced two days later half the dose (200 mg). He had no further complications, except for a grade II mucositis. Bone marrow take was on day +18. Routine weekly CT chest scans don’t reveal any radiological signal of Fusarium sp infection reactivation. Conclusions. At the best of our knowledge this is the first described case of successful secondary prophylaxis with Voriconazole in a patient with previous severe disseminated fusariosis submitted to a stem cell transplantation. Since fusarium infections have a trimodal distribution after SCT (Nucci et al. CID 2004) further cautious follow up will be necessary in this case. But we can conclude that oral Voriconazole seems to be an important new drug that can be safely used for secondary prophylaxis during SCT in patients with previous invasive Fusarium sp infections.

**1441**

**EFFICACY OF PAROMOMYCN AND / OR AZITHROMYCIN IN HEMATOLOGICAL PATIENTS WITH CRYPTOSPORIDIOSIS**

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Cryptosporidium parvum is a widespread parasite of the Apicomplexa genre, with a falc-oral way of transmission and capable of overcoming chlorination of water. Intestinal cryptosporidiosis as a cause of severe diarrhea is not uncommon in underdeveloped countries. Since the 80’s, a rising incidence occurred in Western countries, related to HIV pandemic. There are only isolated cases of the infection in the setting of hematological disease. Diagnosis requires a high level of suspicion and several explorations, often unsuccessful. There is no consensus on therapy, although useful options seem rather unsatisfactory. Oral paromomycin or long-term treatment of Paromomycin, Espiramycin and/or Azithromycin p.o. have been proposed, without certainty of eradication. Case t: 66 yr.-old male. Diagnosed of myelodysplastic syndrome, AREFB 5%, with multiple...
infectious episodes. In March-02, he was admitted to Hospital with fever, loss of weight (12 kg), diarrhea with >10 deposits/day, of liquid orange stools devoid of blood, tenesmus and abdominal tenderness with peritoneal signs. X-ray of abdomen showed diffuse dilatation of gut. Abdominal ultrasound scan and TAC revealed scarce free liquid and thickening of colonic wall. Colonoscopy: replacement of normal mucosa by multiple nodules, resembling sessile polips; a biopsy of one of them was informed of ‘minimal inflammatory changes’. Conventional microbiologic studies rendered no result (cultures, C. difficile toxin, serologies, search for virus and parasites). Specific search for C. parvum (modified Ziehl’s stain) was positive in 3 samples. The CD4+ lymphocyte count was 400/µL. Therapy: paromomycin, 1 g p.o. b.i.d. and diet supplemen-
tation with Lacto-bacillus sp. Resolution of diarrhea, a significant weight gain and improvement in performance status was attained in the following weeks. Case 2: 18 yr.-old male. Diagnosed of Hodgkin’s disease, NS-subtype, stage IV-B, refractory to several lines of chemotherapy, including BEACOPP, ESHAP/MINE and a gemcitabine ‘based scheme. In April-05, he was admitted to Hospital because of protracted fever, diarrhea with green liquid stools, loss of 4 kg in a single week, diffuse abdomi-
nal pain, vomiting and tenesmus. Conventional microbiobiologic studies were also inconclusive. Considering the former case, we asked again for a C. parvum search in stools, which was clearly positive. Therapy with azithromycin (5 days) and paromomycin (14 days) was undertaken, after which diarrhea and fever, as well as the other symptoms disap-
peared in this period; complete clearance of parasite cysts in stools could be demonstrated. A significant recovery of nutritional status was also accomplished. 1. Conventional methods for detecting parasites in stools may not detect C. parvum. This protozoan must be suspected when no diagnosis can be drawn after a complete set of explorations, and an intentional search with specific stains. Although nitazoxanide has been approved for Cryptosporidiosis, this drug is not available in Spain yet. We believe that this simple combination, i.e.: azithromycin, par-
omomycin and diet supplementation is a suitable option for an, otherwise emaciating, unusual form of infectious diarrhea in hematological patients.

1442
ATYPIcal EOSINOPHIL DISTRIBUTION OBSERVED IN PATIENTS WITH MALARIAL INFECTION WHEN USING SYSMEX XE-2100
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1Chung-Ang University, SEOUL, South-Korea; 2Ulsan University Seoul Asan Hospital, SEOUL, South-Korea

Backgrounds. The incidence of malaria has been increasing in civilian population and the prevalent area being more wider in Korea. Malaria must be recognized promptly in order to treat the patient in time and to prevent further spread of infection in the community. Aim. Malaria can be suspected based on the patient’s symptoms and the physical findings at examination. However, for a definitive diagnosis to be made, labora-
tory tests must demonstrate the malaria parasites or their components, which are time consuming and need expertise. As it is likely that gener-
al screening tests like a complete blood cell count are always undertak-
en for patients who present with pyrexia, it can be expected that attention to any abnormalities found in automated hematology analyzer can decrease a delay in the diagnosis of malaria if such a diagnosis was not initially considered.

Methods. Hematological analysis using Sysmex XE-2100 (TOA med-

ical Electronics, Kobe, Japan) and Advia 120 (Bayer Diagnostics, Tarry-
town, NY, USA) was performed on samples positive for malarial para-
ite. Results. We found 3 peculiar patients with P. vivax malaria who had pseudoeosinophilia determined only when using Sysmex XE-2100. Although eosinophilia of 5.4%-24.3% was found in 3 patients when measured by Sysmex XE-2100, eosinophilia was not found either when measured by Advia 120 or read by microscopy. As a result of reviewing the scattergram generated by Sysmex XE-2100, atypical eosinophil distri-
tubation was placed more closely to the neutrophil distribution than typical eosinophil distribution in the WBCs scattergram (Fig. 1). This atypical eosinophil distribution was due to the presence of hemozoin-
containing neutrophils. It was concluded Sysmex XE-2100 analyzer showed erroneous high eosinophil counts. So, it is advisable that reading the WBCs scattergram to find a certain hematolog-
ic abnormality such as atypical eosinophil distribution as a result of hemozoin-containing neutrophils may contribute to the diagnosis of malaria especially for patients unsuspected.

1443
USEFULNESS OF THE VORICONAZOLE PLASMA LEVELS MONITORING IN HEMATOONCOLOGICAL PTS TREATED BY ORAL FORM OF THE VORICONAZOLE
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Backgrounds. Voriconazole, a new azole antifungal agent, is widely used in hematoooncological pts. Even the bioavailability of the voricona-
ole oral form reaches 90%, several situations can lead to the impair-
ment of the drug absorption. Even a lot of discussions about usefulness of the voriconazole plasma levels monitoring, we decided to monitor level-
es of this drug in our pts to confirm adequate absorption in these severely ill pts. Methods. In all pts treated with oral voriconazole from 8/2005 to 2/2006 steady-state trough plasma voriconazole levels were measured using an HPLC assay. Results. 49 samples from 22 pts were tested. Pts had drug levels checked once (n=6), twice (n=7) or thrice (n=7) 4-8 days (median 11) after starting voriconazole or dose modification. Mean and median plasma levels were 1,1 and 0,75 microgram/ml (range: < 0,2 - 5,41 µg/ml). 19 samples (59%) from 10 pts (45%) were < 0,5 microgram/ml (possibly below the in vitro MIC90 for Aspergillus sp.), and 12 samples (24%) from 7 pts (52%) were < 0,25 µg/ml (possibly below the mean in vitro MIC90 for Candida sp. in our dept.). Potentially impaired absorption of the voriconazole (due to worsened intestinal peristalsis or aplication through NG tube) or using of reduce dose of voriconazole as a reason for lower drug steady-state plasma levels were indentified in 7 of 12 samples (58%) with voriconazole plasma level < 0,25 and in other 2 of 7 samples (29%) with voriconazole plasma level between 0,25 - 0,5. Interestingly, in 5 of 12 samples (42%) with voricona-
ole plasma level < 0,25 and in other 5 of 7 samples (71%) with voricon-
azole plasma level between 0,25 - 0,5 the reason for the insufficient drug level in plasma were not identified. Hepatic CYP2C19 genetic polymor-
phism with differences in drug metabolisation can be the possible expla-
nation. The dose of oral voriconazole was increased in 3 pts, that leads to drug steady-state plasma level increase with mean 2,47 microgram/ml. Conclusions. Voriconazole plasma levels after the use of oral form of the drug in hematoooncological pts vary significantly and plasma levels mon-
toring can help to clinicians to confirm achievement of the therapeutic levels of voriconazole especially in pts with gastrointestinal impairment. This approach needs further evaluation.

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1444
JC PAPOVAVIRUS LEUCOENCEPHALOPATHY AFTER TREATMENT WITH CHEMOTHERAPY AND RITUXIMAB
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Background. Progressive multifocal leucoencephalopathy (PML) is a rare demyelinating infection of the central nervous system caused by the JC papovavirus usually seen among immunocompromised patients. The most common underlying immunosuppressive illness is AIDS. Howev-
er, PML may be seen among patients with lymphoproliferative disorders and immunosuppression induced by chemotherapy. Recently, an asso-
ciation between PML and rituximab with autologous or allogeneic trans-
plantation has been discussed. Aims. We report the case of a woman with a mantle cell lymphoma who developed PML after a combination of chemotherapy with rituximab. Methods. A 67 year old woman was
diagnosed with mantle cell lymphoma because of splenomegaly and hy- peri eosinophilia on aspirate testing. Staging shows a stage IV with bone marrow involvement. The patient was treated with a combination of rituximab (375 mg/m² D1) and chemotherapy with standard CHOP: cyclophospahmide 750 mg/m² D1, doxorubicin 50 mg/m² D1, vincristin 2 mg D1, and oral prednison 100 mg D1 to D5 given in 3-week cycles. She received eight cycles of treatment. Evaluation after the eight cycles showed a condition of complete remission. One month after the last chemotherapy, the patient presents rapidly psychiatric disturbances with speech dysfunction and paranoia delirium. PML was suspected after magnetic resonance imaging with frontal and temporal leucoencephalopathy. JC viral DNA was detected in the cerebrospinal fluid. HIV serology was negative. Low level of CD4, CD8, lymphocytes and NK cells were noted. A treatment with cidofovir was started. Two months after the beginning of symptoms, neurological disturbances were stable. Results. A few cases of PML were recently described in patients who were treated with chemotherapy, transplantation and peritransplantation rituximab. A direct association between rituximab and PML remains speculative. Moreover, the patients reported were often in relapse, heavily pre-treated. Our patient is, to our knowledge, the first case of PML after a combination of CHOP with rituximab, in first induction procedure. Conclusions. Unusual viral infections were recently described in patients treated with high dose chemotherapy and rituximab. Although the contributory role of rituximab remains speculative, our additional case highlights the need for an accurate surveillance, even in patients not heavily pre-treated, in first induction with CHOP and rituximab.

1445 VARIEABLE RESPONSE TO CURRENT TREATMENT OPTIONS IN HYPEREOSINOPHILIC SYNDROME (HES)-A SINGLE CENTRE EXPERIENCE
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Backgrounds. Hypereosinophilic syndromes (HES) refers to a het-
erogenous group of disorders characterised by marked blood eosinophilia (>1500/cu mm) and tissue eosinophilia (lasting for more than 6 months), in the absence of other etiologies for eosinophilia, resulting in end organ damage. HES may be a reactive condition or a chronic myeloproliferative disorder with evidence of clonal prolifer-

ative. Reactive eosinophils are due to release of cytokines (IL-3 IL-
5,GM-CSF etc) and the common causes are parasitic (fielminthic) infec-
tions, allergic diseases, vasculitides, drug reactions and malignancies. Clonal eosinophils are those in which the eosinophilia is a part of a clonal haematological malignancy, which is very often associated with the fusion gene FIP1L1-PDGFRα which causes the generation of a con-
stitutively active Tyrosine Kinase. Several visceral complications like cardiomyopathies, nervous system involvement (e.g. paraparesis, cere-
bral infarction, eosinophil meningitis etc) are often fatal illnesses. Treatment modalities for HES includes corticosteroids, chemothera-
paetic agents (hydroxyurea, cyclophosphamide vincristine) and α-interferon. Newer treatment modalities including tyrosine kinase inhibitors (Imatinib, and nilotinase) and monoclonal anti-IL5 antibodies are now available. Patients carrying this fusion gene respond well to the Tyrosine Kinase Inhibitor Imatinib. Some patients with HES, that are negative for this fusion gene may also respond to Imatinib, sug-
gestig that in such cases other Tyrosine Kinases may be dysregulat-
ed. Aim. Retrospective review of the variable response of 5 patients with HES (over a period of 6 months), to current treatment modalities. Methods. The 5 patients (4 Male, Female; age range 37-80 yrs; mean age 56 yrs) presented with eosinophilia in the range 2600-12,000/cumm. A response to treatment was defined as Eosinophil count < 1500 /cumm or Eosinophil count < 5% of the total leucocyte count in the periph-
eral blood. Follow up of 3 patients was available after Imatinib as initial treatment. 1 patient initially had Methyl Prednisolone followed by Imatinib and 1 patient (aged 80 yr) was treated with Hydroxyurea initially. Results. Two of the four patients receiving Imatinib responded to it. Of the 2 patients not responding to Imatinib, 1 responded partially to Hydrox-
yurea and the other did not respond to monotherapy with steroid or α-interferon. However the latter eventually responded to a combina-
tion of steroids and α-interferon. The patient who had initial treatment with hydroxyurea responded well. Of the 5 patients 1 was equivocal (possibly false positive) for the FIP1L1-PDGFRα fusion gene. Two were negative and two were not tested. Of the 2 that were negative 1 responded to Imatinib (see Table). Summary. Thus response of HES patients to the various treatment modalities is variable and often unpredictable. A trial of Imatinib is worth considering in all cases. In case that are refractory to monotherapy with Imatinib, steroids and α- interferon, a combination of the last two agents may be tried. In dif-
ficult resistant cases of HES, monoclonal anti IL 5 may be tried.

Table 1. Hypereosinophilic syndrome.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Male/Female</th>
<th>Clinical Presentation</th>
<th>Eosinophil Count (at presentation)</th>
<th>PDGFRα Status</th>
<th>Initial Treatment</th>
<th>Response To Imatinib</th>
<th>Agents</th>
<th>Second line Agents</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>37/F</td>
<td></td>
<td></td>
<td>-history</td>
<td>2600</td>
<td>Equivocal</td>
<td>Imatinib</td>
<td>No Prednisolone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>43/M</td>
<td></td>
<td></td>
<td>Lacunar Infarct</td>
<td>12,000</td>
<td>Negative</td>
<td>Methyl Prednisolone (followed by Imatinib)</td>
<td>Yes</td>
<td>Not applicable</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>59/M</td>
<td></td>
<td></td>
<td>3500</td>
<td>Not tested</td>
<td>Imatinib</td>
<td>Yes</td>
<td>Not applicable</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>63/M</td>
<td></td>
<td></td>
<td>Atrial Fibrillation</td>
<td>3000</td>
<td>Negative</td>
<td>Imatinib</td>
<td>No Hydreaures</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>80/M</td>
<td></td>
<td></td>
<td>Alzheimer’s Disease</td>
<td>3500</td>
<td>Not tested</td>
<td>Hydreaures</td>
<td>Not applicable</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

M: Male; F: Female;
gesting a common pathogenesis which involves the cytoplasmic tyrosine kinase JAK2 in these disorders. To evaluate the frequency of JAK2V617F status and possible correlation with clinical features in PV, TE and IM. We studied 59 patients, 29 males and 30 females, median age 52.8 years (range 22-83), diagnosed with MPD. According to PVSG criteria, patients were defined as PV (25), ET (27) and IM (7). At the time of the study, patients were followed up with 52.5 months (range 3.5-307). Genomic DNA was extracted by standard procedures from peripheral blood granulocytes. The presence of the JAK2V617F was determined with the JAK2 activating mutation assay (InVivoScribe Technologies, San Diego, CA) based on BsaXI digestion of a PCR product encompassing the site of mutation. Among PV patients, the JAK2V617F mutation was detected in 20/25 cases (80%), with 14 of them (70%) being heterozygous and 6 (30%) homozygous. Comparison between JAK2V617F patients (group A) and JAK2 wild-type patients (group B) did not reveal significant associations with age, gender, hemoglobin levels and platelet count at the time of diagnosis. In contrast, a trend-association was found between median leukocyte count (group A: 9.8 x 10^9/L vs. group B: 7.4 x 10^9/L (p=0.07) and hematocrit (group A: 57.0% vs. group B: 52.9%) (p=0.08). Spleen enlargement was only observed among mutated patients (3/20, 40%). Also thrombotic events were only registered in the JAK2V617F patients (3/20, 15%). Among patients diagnosed with ET, the JAK2V617F mutation was detected in 20/27 cases (74%) and all were heterozygous. Comparison between ET groups A and B did not reveal significant associations with age, gender, leukocyte count. Instead, a trend-association was found for median hematocrit (group A: 40.7% vs. group B: 38.1% (p=0.07), median hemoglobin levels (group A: 14.9 g/dl vs. group B: 13.1 (p=0.06), median platelet count (group A: 878 x 10^3/µl vs. group B: 1.449) (p=0.09). Moreover, thrombotic events were observed only in group A patients (2/20 (10%), among patients with IM, the JAK2V617F mutation was detected in 2/7 cases (28.6%), one heterozygous and one homozygous. The IM JAK2 V617F mutation homozygous patient had in the history a thrombotic event. In our series the JAK2V617F mutation was a very frequent bronchial abnormality in PV (80%) and ET (74%) patients, while it was detectable in a smaller proportion of IM patients (28%). The homozygous JAK2V617F status was only found in PV (6/20) and in IM (1/2), while it was never detected in ET patients.

**1448**
EVALUATION OF THE BRITISH SOCIETY FOR HAEMATOLOGY CRITERIA FOR THE DIAGNOSIS OF POLYCYTHEMIA VERA

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**Backgrounds.** Polycythemia vera (PV) is a myeloproliferative disorder in which the dominant feature is excessive erythropoiesis resulting in a raised red cell mass. Criteria have been established by the Polycythemia Vera Study Group (PVSG), the British Society for Haematology (BSH) and the World Health Organisation (WHO) to diagnose PV and differentiate it from other causes of erythrocytosis. Recently it has been shown that PV is associated with an acquired activating mutation, V617F, of the Janus kinase (JAK2). **Aims.** We retrospectively assessed the diagnostic information of patients with erythrocytosis of all causes (PV, idiopathic, secondary and apparent) against the BSH criteria. We determined if a diagnosis of PV would have been established by these criteria and whether or not this agreed with the diagnosis made by their clinician. We are determining the JAK2 status of this group. **Methods and Results.** The patient sample was drawn from a clinical database. The records of 77 patients who attend Belfast City Hospital with PV (47 patients) and other causes of erythrocytosis (30 patients) were reviewed and relevant information was recorded. Sufficient data was available to apply the BSH criteria to 64 (PV 36, other 28) out of 77 patients (83%). Thirty-five patients met the BSH criteria for a diagnosis of PV and 29 patients did not. There was agreement with the diagnosis established by the patients clinician in 65 out of 66 cases. Only 1 patient had been diagnosed with PV who did not meet the BSH criteria. This patient met both the WHO and PVSG criteria for PV. To date the JAK2 V617F mutation has been demonstrated in 29 of 31 tested patients with PV and in 1 of 12 patients with erythrocytosis of other causes.  **Conclusions.** We concluded that the BSH criteria for the diagnosis of PV were easily applied, sensitive and specific. Results of V617F JAK2 mutational analysis are consistent with previous findings and support the suggestion that this should be incorporated into the initial evaluation of patients with erythrocytosis.

**1449**
RESPONSE TO IMATINIB IN A FIP1L1-PDGFRα NEGATIVE HYPEREOSINOPHILIC SYNDROME(HES) PATIENT WITH STROKE AND INFECTIVE ENDOCARDITIS

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**Backgrounds.** The Hypereosinophilic syndromes are a rare haematologic disorder characterized by eosinophilia (>1.5 x 10^9/L) persisting for more than 6 months in the absence of reactive causes. Recently the FIP1L1-PDGFRA Fusion gene has been identified in 50% of cases of HES. This fusion gene is a constitutively activated tyrosine-kinase. Imatinib mesylate, is a potent selective inhibitor of several Tyrosine Kinases, including PDGF receptors. FIP1L1-PDGFRA fusion gene seems to be another target of this drug.

![Figure 1. HES-Steroids commenced 25/6/5, Imatinib from 29/7/5.](image)
THROMBOCYTOSIS. ETIOLOGIC ANALYSIS OF 1688 HOSPITAL PATIENTS
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Background-Aims-Methods: One thousand six hundred eighty eight hospital patients aged 21 to 86 years with thrombocytosis (defined as a platelet count of more than 450,000/μm³ and below 1,000,000/μm³ in 97% of patients) seen in our hospital over a 15-year period, were studied prospectively for etiological diagnosis. Results: The causes of thrombocytosis were myeloproliferative diseases (6%), malignancy (17%), post-surgery or experiencing tissue damage (massive acute hemorrhage or thrombotic episodes (19%), infections (34%), chronic inflammation (5%), iron deficiency anemia (10%), miscellaneous disease states as cardiac disease, liver cirrhosis, renal failure etc (9%). Thrombocytosis associated with multiple, simultaneous causative factors was seen in 7.9% of cases. Among all hospital patients with infections, sepsis was associated with platelet counts that were not seen in any other infection (P < .0001). Thrombocytosis secondary to infections and malignancy was significantly more common in aged patients. No thrombocytosis-related complications were seen in any hospital patient and none required any specific treatment. Conclusions: Thrombocytosis is a frequent finding in hospital patients. It is due to a variety of etiologic factors and is of significance clinical discriminatory value. It is often due to an acute-phase phenomenon in response to infection, tissue damage, blood loss, or anemia, and is an early sign especially of disseminated, advanced or inoperable malignancy.

PERICARDIAL EFFUSION IN MYELOPROLIFERATIVE DISORDERS
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Backgrounds: Extramedullary haematopoiesis is very common in Chronic Myeloproliferative Disorders (MPDs) and the commonest site of extramedullary haematopoiesis is the spleen and the liver. Unusual sites can sometimes be affected leading to haematopoietic tumours surrounded by a capsule of connective tissue. Such sites include lymph nodes, CNS, skin, pericardium, peritoneum, pleura ovaries, GIT and the lung. Many such cases remain asymptomatic and may be diagnosed incidentally. However in cases where the pericardium is affected cardiac tamponade may result, requiring urgent intervention (pericardiocentesis). Recurrence may be prevented by minor pericardiectomy. In patients with myeloproliferative disorders and increased cardiac silhouette on X-Ray film, with or without clinical heart failure an echocardiogram is recommended in order to identify a possible pericardial effusion. Treatment with radiotherapy have been shown to be effective. Case History: We report a 68 year old man who recently presented with weight loss, mild anaemia moderate thrombocytosis and leucocytosis. A diagnosis of a Myeloproliferative disorder was made 3 years back but he had been transfusion independent. He has background history of atrial Fibrillation which can result in dramatic clinical deterioration; hence an high index of suspicion and an early echocardiogram necessary. Close monitoring in CCU will ensure that urgent intervention (pericardiocentesis) can be undertaken if cardiac tamponade develops.

THE INCIDENCE OF THROMBOTIC EVENTS IN CHRONIC MYELOPROLIFERATIVE DISORDERS
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The thrombophilia due to Chronic Myeloproliferative disorders (CMD) is determinate by modification of rheologic parameters through hyperviscosity, the perturbation on thrombocyte function and the perturbation of the cytokines secretion. The thrombotic events associated to CMD are the result of the thrombophilia feature with the quality of medical care. This is the reason for the analysis of the 136 subjects diagnosed in our Clinics with CMD between 2000 - 2005. We diagnosed in 52 subjects Polycythemia Vera (PV) (32,3%), in 32 subjects Essential Thrombocythemia (ET) (23,52%), in 26 subjects Agnogenic Myeloid Metaplasia (AMM) (19,1%) and in 26 subjects Chronic Myeloid Leukemia (CML) (19,1%). There were diagnosed thrombotic events in 49,26% subjects: recurrent cerebral thrombosis in 28 subjects (20,58%); 34,46% in PV, 18,75% in ET, 7,79% in CML. Recurrent thrombembolitis in 8 subjects (5,88%); 12,5% in ET, 7,69% in AMM and 3,84% in PV. Superficial thrombembolitis: 2 subject in ET (6,25%); central retinal vein thrombosis: 2 subject in CML (7,69%); Disseminated intravascular coagulation: 2 subject in ET (6,25%); Spleen infarction: 2 subject in CML (7,69%); Portal vein thrombosis: 4 subjects (2,94%); 7,69% in AMM and 6,25% in ET; Arterial and capillary thrombosis 8 subject (6,25%); 11,53% in PV, and 6,25% in ET; Necrotizing purpura: 1 subject in ET (6,125%); Heart infarction: 9 subjects (6,61%); 15,625% in ET, 7,7% in PV; Mesenteric infarction 1 subject in ET (3,125%). The frequency of thrombotic events was 75% in ET, 61,5% in PV, 23,07% in CML and 15,88% in AMM. Conclusion: The thrombotic events are an important risk factor in CMD, especially in ET and PV. The thrombotic events developed before the diagnostic of CMD and 25% after this. This impose to facility the access to modern therapy: Erythropothesis, Anagrelide, Glivec (imatinib), α interpheron and to consider the primary and secondary thrombophilia as major risk factor for cardiovascular disease.

WHY WE HAVE NO WORKING SYSTEM OF ELECTRON CASE HISTORY UNTIL NOW? EFFORTS TO DEVELOP THE NATIONAL STANDARD
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National Center for Hematology, MOSCOW, Russian Federation

The computerized case history system allows the collection of medical information from multiple sources, integrated presentation, fast search, simultaneous availability to several participant of medical process etc. It is efficient for patient follow-up, consultation, and transfer as well as for analysis of clinical trials. The subsidiary role of information systems in medical process partly the result of insufficient attention is given to the question of the official status of electronic personal medical records and documents. In the majority of hospitals such systems are used only for preparation and printing of medical documents, which signed by ink, participate in traditional medical document circulation. Case history or research forms of clinical trials shelved in storage and if could to be retrospectively analyzed, only with huge efforts. It seems, that most of pharmacological companies, conducting clinical trials are...
not interested in direct collecting data in electron database, because such system able to provide real audit of study procedures and conclusions, practically impossible with paper form collection. Use of the electronic medical document and electronic archives demands to provide: - An invariance and reliability during all period of storage; - A regulation of the access to and confidentiality; - Personification (an opportunity to define the record holder and the record owner) - analogue of the signature on the traditional document). Concerning the traditional medical documentation a lot of normative were developed. Electronic personal medical records needs the development of standards providing their legal status and an effective utilization in medicine and public health services. It’s so happened in Russia, that the project 'The National Standard of Medical case history' were presented by the National Center for Hematology.

1454
ERDHEIM-CHESTER DISEASE: TREATMENT WITH INTERFERON-α
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Erdheim-Chester (EC) is a rare non-Langerhans’ cell histiocytosis of unknown etiology. This rare illness has a particular tropism for connective and adipose tissues. There are typical radiographical and pathological features, which can lead to the diagnosis, but the clinical spectrum ranges from a focal asymptomatic process to a multisystemic, rapidly fatal, infiltrative disease. The entity is defined by a mononuclear infiltrate consisting of lipid laden foamy histiocytes that stain positively for CD68, and negatively for CD1a, CD1a, and S100. The differential diagnosis includes Langerhans’ cell histiocytosis, metabolic disorders, and malignancies. The outcome of patients is worse than that for Langerhans’ cell histiocytosis with about 60% of patients dead after a mean follow-up of 32 months, whereas only 9% of patients with the latter disorder have succumbed after a median follow-up of 4 years. Corticosteroids, chemotherapy, surgical resection, and radiotherapy have been used to combat EC disease and there is no consensus concerning the best treatment. A recent study showed good outcome of 3 patients with advanced disease but without pulmonary and cardiac involvement treated with interferon-α (IFN-α). The aim of this study is to present an advanced EC case with cardiac, pulmonary, bone, retro-orbital and retroperitoneal fibrosis that showed bad outcome of the cardiac function with IFN-α therapy. We evaluated a 48-year-old woman with a 2-year history of striking exophthalmos. Her previous history included retroperitoneal fibrosis with unknown cause and obstructive renal impairment that led to chronic kidney failure. The ophthalmological examination revealed a Hertel exophthalmometry measurement of 22mm (normal 12-20mm). Computed tomography of the orbits showed massive infiltration. Retro-orbital mass biopsy was consistent with EC. Prior to treatment the exams showed 46% of heart ejection fraction on the left ventricle, lung function with a mild restrictive ventilation defect and a simetrical involvement of the lung parenchyma. On the other hand, IFN-α is known to induce adverse effects such as cardiac dysfunction, cardiomyopathy, various kinds of arthritisms, and sudden cardiac death, although clinical trials which evoke these cardiac events are not documented. This aggravation can be useful. The obstetrical team evaluated maternal and neonatal pairs during the pre-partum period in the maternity wards collaborating in the cord blood program. Donors signed informed consent before delivery, during last months of pregnancy. The CB units collected in triple bag system were centrifuged in oval buckets at 3000 g for 12 min at 2°C, ensuring that the bags were well supported to prevent disruption of the buffy coat layer. CB collections were separated into plasma, red blood cells concentrate (RBCC) and buffy coat (BC) containing haematopoietic progenitors with two different devices: Optipress II and Compatom G4. A standard protocol programmed into the Optipress II, together with the standard backplate for BC preparation was used to process the CB units (n=27). The programme was set with the following parameters: BC volume of 40 ml, a BC level of 5.5 and a force of 25. Program CB1 in Compatom G4 device was empirically developed to reach a BC volume of 41 ml (n=31). Monitoring the TNC, RBC, CD34+ cells and CFU content in both pre-process and post-process CB units assessed the volume reduction process during the development phase of the study. Results. Table 1 shows the results of the development phase of the study. When the two devices were introduced into routine, lymphocytes recovery (79.6±10.9% for Optipress II and 77.8±7.5% for Compatom G4, p<0.001) and red blood cell depletion (55.6±16.1% for Optipress II and 51.4±14.9% for Compatom G4, p<0.005) were significantly better for CB units processed with Optipress II. TNC recovery was similar for both methods (78.5±7.8% for Optipress and 78.2±7.2% for Compatom G4, p=ns). Conclusions. Compared to Optipress II, volume reduction with Compatom G4 device allows worse lymphocyte recovery and RBC depletion of cord blood units.

Table 1.

<table>
<thead>
<tr>
<th></th>
<th>Optipress II</th>
<th>Compatom G4</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNC Recovery (%)</td>
<td>77.5±10.3</td>
<td>79.8±9.3</td>
</tr>
<tr>
<td>CD34 recovery (%)</td>
<td>116.6±81.9</td>
<td>85.0±13.6</td>
</tr>
<tr>
<td>CFU recovery (%)</td>
<td>93.6±26.8</td>
<td>88.7±41.1</td>
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</tbody>
</table>

1456
A VERY SMALL POPULATION OF CELLS EXPRESSING THE CD133 HEMATOPOIETIC STEM CELL ANTIGEN EXISTS IN HUMAN ADULT SUBSTANTI A NIGRA AND STRIATUM BRAIN TISSUES
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Numerous animal studies have demonstrated the presence of neural stem cells in the mammalian forebrain. Recently, the CD133 haematopoietic stem cell antigen has been identified in foetal human brain tissue, human focial cortical dysplasia, prematures infants cortex and in paediatric brain tumours. To date, it is unclear whether these stem cells exist in human adult brain tissues. The aim of the present study was to evaluate the presence of CD133 positive cells in the various areas of postmortem human midbrain and hindbrain tissues including the substantia nigra, stratum, medulla and pons. The immunocytochemical staining with anti-CD133 epitope 1 (clone 5H3, Miltenyi Biotech Ltd.) and anti-CD133 epitope 2 (clone 293C3, Miltenyi Biotech Ltd.) revealed the presence of CD133 epitope-2 and not epitope-1 in only two sites: substantia nigra and stratum in post-mortem brain tissue sections from 4 elderly patients purchased from Medical Solution plc. (Nottingham, UK). The CD133 epitope-2 positive cells were oval prolonged in shape and have size of 150-172 μm². The CD133 positive cells in the substantia nigra were larger than those in the Striatum. The lack of any expression of CD133 epitope-1 in adult brain is in line with previous PCR-studies. Also, we investigated the presence of any expression of the OCT-4 embryonic antigen in the same adult brain tissue sections. Only a small population of cells expressing OCT-4 was found only in the same two sites: Substantia Nigra and Striatum as shown in the figures. Since in the substantia nigra of the midbrain, degeneration of dopaminergic neurons is responsible for the debilitating motor dysfunction in patients with Parkinson’s disease. Further studies are warrant-
HLA-DR. Still remains to be proved whether this expression has any functional antigen-presenting role. CD33 is a marker of immature and has a regulatory effect in the maturation of myeloid cells, the monocytic/macrophages function and the production of dendritic cells. Its expression on high percentage of granulocytes could be due to: (a) increased mobilization of granulocytes from bone marrow due to GFs effect; (b) high rate of differentiation, resulting in preservation of immature markers on granulocytes surface; and (c) possible reactivation of CD33 genes due to cytokines effect.

Table 2. Percentage (%) of la + and CD33 + granulocytes in vitro tests.

<table>
<thead>
<tr>
<th>T0h RPMI</th>
<th>RPMI+GM-CSF</th>
<th>T24h RPMI</th>
<th>RPMI+FBS</th>
<th>RPMI+GM-CSF+FBS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>0-2</td>
<td>0</td>
<td>5-20</td>
<td>10</td>
</tr>
<tr>
<td>AML</td>
<td>2-5</td>
<td>0-10</td>
<td>15-35</td>
<td>10-25</td>
</tr>
<tr>
<td>Solid tumors</td>
<td>5</td>
<td>5-20</td>
<td>20</td>
<td>10-25</td>
</tr>
</tbody>
</table>

In most cases those antigens were expressed on granulocytes from the first day of GFs' administration and they were preserved on their surface even 20 days after the final day of GF injection. In vitro tests showed induction of HLA-DR on granulocytes after incubation with GM-CSF (Table 2). Conclusions. According to our data, administration of growth factors induces the circulation of HLA-DR+ granulocytes in peripheral blood. In vitro tests also confirm this finding. In addition, the results from in vitro tests indicate that GM-CSF directly affects granulocytes, inducing synthesis and expression of the antigen-presenting molecule.
changes in the human ε-globin gene reported to date, rather than a single nucleotide polymorphism (SNP), located at the 5' regulatory region of the gene. Aims. To develop a non-radioactive single strand conformation polymorphism (SSCP) approach to screen the human ε-globin gene and its regulatory regions for possible mutations and single nucleotide polymorphisms in normal adult subjects, in order to determine those genotypes which are dispensable for its proper regulation and function. Methods. Peripheral blood was collected from 60 unrelated normal male and female donors, whose age ranged between 25-50 years. Informed consent was obtained prior to the study. Genomic DNA was extracted from peripheral blood leukocytes. Human ε-globin gene coding and regulatory regions were amplified in 5 consecutive fragments, using 25 pmoles of each primer (primer sequence available on request). PCR products were then analyzed, using a non-radioactive (silver-staining) single strand conformation polymorphism (SSCP) analysis. Where needed, temperature has been adjusted (from 4-8°C, increment: 1°C) to improve resolution. DNA sequence analysis was performed using automated fluorescent DNA sequencer (ABI PRISM 310 Genetic Analyzer, Applied Biosystems, CA, USA). Results. Selection of the fragments was done on the basis of analyzing the entire coding and regulatory regions of the ε-globin gene in addition to the majority of intronic sequences. Heterozygous and homozygous cases for the 5'A/HincII SNP were analyzed as positive controls to ensure optimal resolution and detection of the expected nucleotide changes. Apart from the aforementioned SNP in the expected frequencies for the Hellenic population, our mutation screening approach of 120 normal chromosomes from adult individuals yielded no other nucleotide change in all samples in the region analyzed. DNA sequencing of 10 randomly selected cases in all 5 fragments confirmed the presence only of the wild-type allele. A reminiscent of this situation is the human ε-globin genes, which are also mutation-free in their proximal promoter regions. Conclusions. This observation suggests that nucleotide changes in the human ε-globin gene are most likely incompatible with normal erythropoiesis and proper embryonic development. The possibility that mutations or SNPs are present in an 562bp region of intron II, which has not included in our experimental design, cannot be ruled out, although it is less likely as this region is also mostly unaltered in the rest of the globin genes studied.

1461

OUTCOME OF CHILDREN WITH APLASTIC ANEMIA IN A DEVELOPING COUNTRY. A SINGLE CENTRE EXPERIENCE

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Background. Aplastic anemia (AA) is a heterogeneous clinical syndrome, representing a serious challenge for a health system confronted with shortages and with limited experience in bone marrow transplantation. Aims. Given these conditions of treatment we sought to evaluate outcomes of patients with AA treated in our centre. Methods. We retrospectively analyzed the records of 28 patients consecutively admitted in our centre in the period from 1995 to 2005; their mean age was 10.5 years (3 months 22 year) and the sex ratio male/female was 1.33. They all met the criteria for AA: 2- non severe, 2 -severe and 24 -very severe form. For all patients a diagnostic workup had been performed consisting of: quantitative serum immunoglobulin panel, flowcytometry study of lymphocytes, anti-nuclear and anti-DNA test, viral hepatitis, cytomegalovirus, Epstein-Barr virus, HIV-1 and 2 serologies, bone marrow aspirate and biopsy, including histologic and cytogenetic analyses. Three patients fulfilled the criteria for hereditary AA (Fanconi anemia-1, Dyskeratosis congenita-1, familial autosomal dominant form-1 case). 6 cases were connected with selective IgA deficiency and 18 were idiopathic forms. Results. The treatment consisted of: corticosteroid therapy ± G-CSF (4 cases), androgen preparations (12 cases) and cyclophosphorine A (CsA) (17 cases). 10 patients received a standardized regimen of antithymocyte globuline (ATG) and CsA; one patient with very severe form was transplanted with related HLA-compatible marrow. Most of patients (17-60.7%) died: 8 with severe sepsis, 5 with bleeding accident and 1 developed a fatal myeloblastic leukemia. The unavourable outcome characterized 3 patients with hereditary form (except the patients with dyskeratosis congenita), all patients with postinfectious AA and the patient with IgA deficiency. I2 cases with idiopathic AA survived, 8 of them with very severe form, treated during the first 2-4 months of disease with ATG + CsA. Conclusions. AA remains in our experience the hematological disease with the worst prognosis. To assure the accessibility to allogeneic bone marrow transplantation and to appropriate immuno- suppressive therapy is our mandatory future task.

1462

ACUTE LEUKEMIA AND MYELODYSPLASIA REVEALING FANCONI ANEMIA: REPORT OF 6 CASES.

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Fanconi anemia (FA) is a rare autosomal recessive disease characterized by progressive pancytopenia, congenital malformations and predisposition to myelodysplasia (MDS) and acute myeloid leukemia (AML). FA is rarely revealed by AML or MDS. We report 6 cases of patients unknown before with AF and who develope AML and MDS. The ages of patients ranged from 8 to 25 years. The mean age at diagnosis was 11 years. Malformations were present in two cases and consisted of skeletal malformations. Abnormal skin pigmentation were present in 5 cases. AML was noted in 4 cases and MDS in 2 cases. The diagnosis of FA had been proven by chromosome breakage analysis. Cytogenetic analysis showed monosomy 7 in 3 cases and del 6p in one case. In two cases, the therapy was delivered only in 2 cases. The outcome was unfavourable with death in 5 cases. This study suggest to perform systematically a cytogenetic analysis to diagnose FA in childhood AML in tunisian population, which is characterized by its heterogenous ethnic background and by a high rate of consanguinity.

1463

PRETRANSPLANT BONE MARROW MICROENVIRONMENT PLAYS AN IMPORTANT ROLE FOR ENGRAFTMENT AND TRANSPLANTATION OUTCOME IN NON-MYELOABLATIVE STEM CELL TRANSPLANTATION


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Backgrounds. It has been suggested that both bone marrow (BM) microenvironment and hematopoietic stem cells play important roles

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for engraftment and immuno-hematopoietic recovery after myeloablative hematopoietic stem cell transplantation. However, the relevance of BM microenvironment implicated in the engraftment and transplantation outcome after non-myeloablative stem cell transplantation (NST) has not been thoroughly evaluated. Aims. In this study, we tried to evaluate the relevance of BM microenvironment implicated in the engraftment and transplantation outcome after non-myeloablative stem cell transplantation. Method. We evaluated quantitatively the effect of NST BM microenvironment with respect to BM cellularity, BM fibrosis, and presence of multi-lineage dysplasia. In addition, for estimating the iron overloading status, we measured the serum ferritin level simultaneously. A total of 51 patients received an allograft using a fludarabine/busulfan NST regimen incorporating alemtuzumab from a sibling (n=39) or unrelated donor (n=12). The underlying diseases underlying this cohort as follows: acute myeloid leukemia in 21, severe aplastic anemia in 7, myelodysplastic syndrome in 7, multiple myeloma in 6, chronic myeloid leukemia in 4, and non-Hodgkin’s lymphoma in 3 cases. The median age of the recipient was 46 years (range, 18 to 67 years). Although all patients received more than 2 x 10^9/kg of CD34-positive cells during allografting, five patients (9.8%) had graft failure. Pre-transplant poor BM microenvironment was arbitrarily defined as having one of the following parameters: BM cellularity < or = 20%, BM fibrosis (grade = or > 3), presence of myelodysplastic feature (or = or > 2 lineages), and serum ferritin level = or > 1,000 microg/L. Neither of these four parameters was independently associated with graft failure. The patients with serum ferritin = or > 1,000 µg/L (5/26, 19.2%) compared to the cases with serum ferritin < 1,000 microg/L (0/27, P = 0.051). Disease-free survival rate was significantly lower in the cases with iron overloading before NST (p=0.019). When the patients were divided into two groups according to the sum of each parameter (0 or 1, 1 > or = 2), the patients with more than 2 parameters showed higher probability of graft failure (5/24, 20.8%) compared to cases with less than 2 (0/27, P = 0.018). Disease-free survival rate was also significantly lower in the patients with more than 2 parameters (p=0.041). However, there were no significant differences between two groups in the incidence of acute graft-versus-host disease (GVHD) (0 or = or > 2 grade) and chronic GVHD (0 or = or > grade 3). Taken together, we suggest that transplant BM characteristics tentatively reflecting microenvironment function provide valuable information predicting the engraftment and transplantation outcome, including disease-free survival, in NST settings.

1464 SYMPTOMS IN LONG-TERM SURVIVORS OF CHILDHOOD BLOOD CANCER AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION (BMT), HEMATOPOIETIC STEM CELL TRANSPLANTATION (HCT)

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Background. Allogeneic BMT/H SCT improves outcomes in children with blood cancer. However late effects in long-term survivors after BMT/H SCT are understudied. Aims. In this connection the aim of our study was to assess symptoms in long-term survivors of childhood blood cancer after allogeneic BMT/H SCT. Patients and Methods. Eighteen survivors were evaluated at 1-18 years (median, 3 years) after allogeneic BMT/H SCT for acute leukemia (15), chronic leukemia (2) and myelodysplastic syndrome (1). Median age at transplantation (NST) was 11.5 years (range 2 - 21), girls/boys - 11/7. Acute or chronic graft-versus-host disease (GVHT) after BMT/H SCT was observed in 12 survivors. For symptom assessment NJ Children Cancer Symptom Inventory and MD Anderson Symptom Inventory were used in the group younger than 18 yrs at the time of the survey (n=11) and in the group 18 yrs and older (n=7), respectively. The validated Scale was used for symptom assessment in five-year old girl. Results. All the survivors experienced at least one symptom. Twelve survivors (67%) had moderate-to-severe symptoms. The most prevalent symptoms were fatigue and pain (90% survivors); 25% survivors presented moderate-to-severe pain or fatigue. The other prevalent symptoms were lack of appetite (78% survivors, 21% - moderate-to-severe level), sadness (72% survivors, 21% - moderate-to-severe level) and sleep disturbance (56% survivors, 10% - moderate-to-severe level). Other symptoms were shortness of breath, drowsiness, nausea and vision problems. Nine survivors had at least two moderate-to-severe symptoms. Two of them experienced 5 moderate-to-severe symptoms. Among the survivors with moderate-to-severe symptoms 8 survivors experienced acute/chronic GVHD. Conclusions. Our findings demonstrate that more than half of childhood blood cancer survivors experience different pronounced symptoms in long-term period after transplantation. This confirms the importance of symptom monitoring in order to improve/preserve quality of life in long-term survivors of childhood blood cancer after allogeneic BMT/H SCT.

1465 INCIDENCE OF HLA ANTIBODIES IN ALLOGENEIC STEM CELL RECIPIENTS

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The presence of patient-anti-donor HLA alloantibodies can increase the risk of graft rejection in allogeneic stem cell transplantations and a positive crossmatch against donor lymphocytes may be a predictor for graft failure. Therefore evaluation of patients sera for HLA antibodies prior to transplantation is routine in most centres. We additionally focussed on the development of HLA antibodies after HLA fully matched compared to partially mismatched stem cell transplantations and on consequential clinical complications. Sixteen patients who were fully matched and 12 partially mismatched (12/13) with one antibody mismatched allogeneic stem cells were screened for HLA class I and II antibodies by ELISA based methods at the time of registration, prior to and in monthly intervals subsequently to transplantation. Donors were tested for HLA antibodies once prior to transplantation. B- and T-cell crossmatches based on complement dependent cytotoxicity (CDC) were done in 18 cases, depending on the development of HLA antibodies. The mean observation period was 54 (28-202) days post transplantation. Two patients and one donor had HLA antibodies before transplantation, which were not directed against transplanted or host antigens respectively, as these transplantations were fully matched. All lymphocytotoxic crossmatches were negative. Preformed antibodies were detectable up to day +60 after transplantation. Five patients developed de novo HLA alloimmunization between day +14 and day +112. Three of them had received fully matched and two got HLA mismatched grafts. All alloantibody specificities were unrelated to host or graft HLA antigens. Relaps was reported on 6 of the not immunised and one of the immunised patients. There was no association between the development of HLA antibodies and acute or chronic GVHD. The only patient who rejected his graft had no HLA alloantibodies at all. Among our patients HLA antibodies did not raise a problem in transplantation schedule. Nevertheless we routinely go on evaluating all patients for HLA antibodies whenever a donor search is started in order to define unacceptable HLA matches. Immediately before HLA mismatched transplantations patients and donors are screened for antibodies against non shared HLA antigens, as donor cells of sufficient viability for crossmatching are not always available. After stem cell transplantations graft-host recognition did not seem predominantly responsible for HLA alloimmunisation. Patients who developed HLA antibodies was no clear predictive factor for GVHD, relaps or graft rejection.

1466 ALLOGENEIC STEM CELL TRANSPLANTATION FROM A DONOR WITH MOSAIC TURNER SYNDROME

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Backgrounds. Turner syndrome (TS) is a genetic disorder affecting 1/2000-2500 liveborn females caused by the complete or partial absence of one of the X chromosomes, frequently accompanied by cell line mosaicism. In rare cases of stem cell transplantation (SCT) the only available HLA-matched donor could be a female with mosaic Turner syndrome. As disturbances in the immune system have been detected in TS, X lymphocytes may show an increased sensitivity to immunosuppressive therapy throughout the post-transplant period, possibly compromising the patient’s immune capacity. Aims. The aim of this study is to report a case of allogeneic SCT from a donor with mosaic TS in order to evaluate the outcome of this transplantation. Patient and Results. A 47-year-old male with AML-M1 received a peripheral blood SCT from his 54-year-old 1 HLA-A antigen matched sister in whom the cytogenetic analysis revealed a constitutional mosaic Turner syndrome.
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turbed balance of matrix synthesis and proteolytic degradation that
gate whether serum markers for collagen metabolism reflect the dis-
sion the present report demonstrates no difference among poly-
platelet counts, haemoglobin, WBC counts and age. No difference was
stastically significant correlation between serum MMP-9 levels and
by a commercial quantitative sandwich enzyme immunoassay. Serum
statically significant (p=0.086). In the patient group and in the control group we found no sta-

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Backgrounds. Myelofibrosis is a clonal myeloproliferative disorder char-
acterized by splenomegaly, abnormal deposition of collagen in the bone
arrow, extramedullary haematopoiesis, dacrocytosis and leukoery-
phloblasts in the blood smear. The development and prolongation of fibrosis
mediated by complex network of several cytokines. These cytokines
mainly include transforming growth factor α, basic fibroblast growth fac-
tor, vascular endothelial growth factor, platelet factor 4, calmodulin and
necrosis factor α, TNF-α. Aims. Based on role cytokines in myelofi-
brosis, we present an atypical case of leukemic transformation in
myelofibrosis associated with diffuse osteolytic lesions and extremely
elevated sera TNF-α and LDH without disturbance in parathormone in a
49-year-old female that firstly developed malaise and abdominal pain at
first visit. Results. The laboratory analyses showed decrease in hemo-
hematopoietic tissue by monocytes and mature granulocytes and is
increased fibrosis. Matrix metalloproteinase-9 (MMP-9) is produced in
and the fibrotic tissue deposition. Angiogenesis shares common mech-
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1468
ELEVATED TNF-α AND LDH WITHOUT DISTURBANCE IN PARATHORMONE ASSOCIATED WITH
DIFFUSE OSTEOLYTIC LESIONS IN LEUKEMIC TRANSFORMATION OF MYEOFIBROSIS
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Summary/Conclusions. We postulated that elevated

FIP1L1/PDGFRα-NEGATIVE CHRONIC EOSINOPHILIC LEUKEMIA SUCCESSFULLY
TREATED WITH IMATINIB CASE REPORT
T.S. Sztokowski, Z. Pikałowa, J. Straslawska, B. Katrincsakova, J. Hanzlikova, E. Faber, M. Jarosova, K. Indrak
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Background. Recognition of tyrosin kinases contribution to pathogen-
esis of idiopathic hypereosinophilic syndrome/chronic eosinophilic
leukemia (IHE/CEL) and imatinib treatment tended to rapid improve-
ment in prognosis of significant part of IHE/CEL patients. Imatinib is
especially effective in FIP1L1/PDGFRα- (F/P) positive patients. Approx-
imately 40% of responding patients lack the F/P fusion gene, suggesting
other tyrosin kinase influence. Patient and methods. Authors describe case

1467
LEVELS OF METALLOPROTEINASE-9 (MMP-9) IN PATIENTS WITH POLYCYTHAEMIA VERA
S. Theodoridou1, T. Vyzantiadi,1 V. Perifanis,1 E. Mandala1, I. Venizelos1, S. Vakalopoulou1, E. Leukou1, E. Vlachaki1,
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Summary/Conclusions. Our patient manifested osteo-
porosis with a pathologic fracture of the L2 vertebra and CMV reac-
tion. Nineteen months later, the patient is in fairly good general con-
dition. He has limited cutaneous GVHD, suffering mainly from his
orthopedic problem. Summary/Conclusions. Our patient manifested osteo-
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1465
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SYNDROME/CHRONIC EOSINOPHILIC LEUKEMIA (IHE/CEL)
J. Hanzlikova, E. Faber, M. Jarosova, K. Indrak

11th Congress of the European Hematology Association

1466
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other tyrosin kinase influence. Patient and methods. Authors describe case
report of IHE5 patient diagnosed in 1998. Serious organ damage developed and 2 grades required treatment initiation. Corticoids were quite ineffective as well as cyclosporin A was. Cyto genetic examination of bone marrow cells revealed in 49% of examined metaphases karyotype 45,X,-Y. Fluorescence in situ hybridization (FISH)-based strategy was used to detect F/P in bone marrow cells. Using bacterial artificial chromosome (BAC) probes fusion of F/P was not revealed. Considering the staging, the diagnosis was idiopathic myelofibrosis with contamination and hematopoietic finding mimicking transformation was started. Matinab in dose of 100 mg daily was administered despite the F/P negativity. Results. Eosinophils fully disappeared after 6 days of the therapy. Complete hematologic remission was achieved after 2 weeks. Cytogenetic response was assessed after 3 months of treatment. Conclusions. The case of IHE5/CEL presented with the karyotype 45, X, -Y/F/P negative and imatinib-sensitive has not been published yet. Identification of imatinib-sensitive target structure responsible for the disease development is a challenge to future research.

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1470 MIXED IMMUNODEFICIENCY, ATYPICAL MYCOBACTERIA AND MEYELOFIBROSIS
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Background and Aims. Myelofibrosis can be idiopathic (a chronic myeloproliferative syndrome) or secondary to many kinds of insults, as a reaction to malignancy, infections, endocrinopathies, autoimmune diseases and hematopoietic diseases. Pancytopenia and hepato-splenomegaly are frequent, and only reversible when the secondary injury can be treated. The authors present a case of myelofibrosis diagnosed in an 11 months old boy; later it was discovered to be secondary to atypical mycobacteria and quadruple antibacterial therapy for one year reversed the clinical status. Methods/Case History. A Caucasian male infant 4 months old presents with anaemia and pneumonia with pleural effusion. Three months later, he has a peri-anal abscess and presents with hepatosplenomegaly and pancytopenia. The analysis revealed Hb 8,0g/dl (with presence of erythroblasts, frank anisopoikilocytosis, and dacrocytes), leukocytes 1500/mm³ (absolute neutropenia 150/mm³), left shift and basophilia), platelets 65000/mm³; LDH 632 U/L. Bone marrow smear and biopsy showed severe myelofibrosis (without cytogenetic alterations). The thorough studies also demonstrated a mixed immunodeficiency (hypogammaglobulinemia, lymphocytopenia, inversion in the relation CD4+/CD8+) and an auto-immune phenomenon (presence of auto-antibodies against platelets and anti-granulocytes). The boy was given G-CSF on alternate days (10 µg/kg), immunoglobulin on a mesonal basis and co-trimoxazole as prophylaxis for infections. With two years and 6 months old, he had already pericarditis, otitis with tympanic perforation, cutaneous mycosis, several gastro-enteritis and two pyelonephritis. Although he presented palpable lymph nodes in several areas and 6 cm beyond costal grid, his physical and somatometric development were normal for his age. The clinical status degrades with a growing spleen (surpassing the iliac crest) and worsening pancytopenia (Hb 6,9g/dl; leukocytes 1000/mm³; platelets 50,000/mm³), with muco-cutaneous blood discrasia and signs of extra-medullar hematopoiesis on both kidneys. As he has no brothers, a search was begun in the international panel for bone marrow transplantation. It was stopped by the decision for splenectomy (4 years old). The spleen showed numerous tuberculous granulomes without caseous necrosis and although the research for typical mycobacteria was negative, he began quadruple therapy (isoniazide, rifampicine, pirazinamide and ethambutol). After splenectomy, there was normalization of haemoglobin and of platelet number; the leukocytes didn't rise as much (2000-3000/mm³), maintaining absolute neutropenia. A bone marrow smear revealed recuperation of the three hematopoietic series. Immunoglobulin therapy was suspended 2 years after splenectomy, with co-trimoxazol and penicillin prophylaxis, at 11 years old, he doesn't have a significative number of bacterial infections. Analytically, he presents Hb 13,7g/dl; leukocytes 2000/mm³ (neutrophils 120/mm³), platelets 662.000/mm³. Conclusions. Although one of the causatives induced for secondary myelofibrosis is granulomatous disease, there aren't any published cases reporting myelofibrosis secondary to typical or atypical mycobacteria, whether in immunocompetent or immunodepressed individuals. Also it is not usual to see extra-medullar haematopoiesis on both kidneys, causing enlargement and loss of differentiation cortico-medullar. Finally, both splenecotomy and anti-bacillary therapeutic were decitive in the regression of the clinical state, being the remaining neutropenia a manifestation of the immunodeficiency syndrome.

1471 PLATELET FUNCTION EXAMINATION IN ESSENTIAL THROMBOCYTHEMIA
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Backgrounds. Basic diagnosis of essential thrombocythemia is proved by estimation of elevated platelets count and corresponding findings of bone marrow megakaryopoiesis in bone marrow with contemporaneous inclining their reactive changes. The therapy is mainly focused on correction of high platelets count in the blood. The treatment is different in young and elderly patients, in cardiovascular or thrombotic risk and non-risk patients. However, the treatment influences not only the count, but also the function of platelets and so can lead to influencing of clinical symptoms. The function of platelets is not currently investigated before drug administration and in the course of the treatment. Aim of the study was to evaluate clinical and laboratory importance of platelet functional characteristics in essential thrombocythemia. Methods. 30 patients have been included in our observation and we performed (beside the basic laboratory test) - platelets aggregation according to Born, PFA-100 examination and flow-cytometric estimation of CD36, CD42a, CD61, CD62, CD63 markers. The laboratory testing was done before and six months after the treatment. (The platelet aggregation was tested using ADP in two concentrations, colagen and caticonic propylgalat as inductors, and three parameters - percentil of aggregation, slope and desaggregation rate). Results. The decreasing of aggregation response (in 16 cases after all inducers used) was not accompanied by statistically significant changes in other examinations of platelet function tests. Moreover, there were not observed statistically significant changes in repeated examinations after six month of the treatment. There was even no correlation between functional examination of the platelets and clinical symptoms of the disease. Conclusion. Functional disorder of the platelets seems to be the part of clinical findings of the disease, but does not correspond with biological activity of the disease or with its clinical symptoms and/or with the answer to the therapy. Although, the treatment (especially with acid acetylsalicylic-ASA) can widely modify platelet function, it was not observed to be significantly different in our ASA-treated vs. ASA-untreated patients.

1472 POLYCYTHEMIA VERA INITIALLY DIAGNOSED AS ESSENTIAL THROMBOCYTHEMIA
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Backgrounds. The differentiated diagnosis as part of the myeloid proliferative chronic disease Ph- remains afterwards difficult, in spite of the numerous tests. The remission of the disease is a challenge to future research. 3 cases from the three of them presented more than 12.000/mm³. The spleen varied between 1, 5 and 2, 5 cm under the costal board. At the beginning, the value of Hb and Ht did not allow the diagnosis of PV and the high count of the platelets (650 0000, 74 0000 and 82 0000/mm³) imposed the diagnosis of ET. The bone marrow examination was applied (after 2002) for only a patient, releasing on bone marrow biopsy: hypercellularity with hyperplasia of all marrow elements, with left deviation of the erythrocyte clusters, a polymorph megakaryocytic aspect, and that's why it was considered unclassified myeloproliferative disorder. The evolution of those three patients was in 8-18 months towards a classic PV with high levels of Hb and Ht who needed phlebotomy. Conclusions. At the point of prognostic and the therapeutical view of the integration in TE or PV has a low clinical importance. The differentiation as part of the myeloid proliferative chronic disease Ph- remains afterwards difficult, in spite of the numerous tests. The remission of the disease is a challenge to future research.
study of the marrow and the evaluation of the new biological markers can chunk the diagnosis even at the beginning of the diseases.

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SUCCESSFUL TREATMENT OF CHILDHOOD IDIOPATHIC MYELOFIBROSIS WITH STEROID
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Idiopathic myelofibrosis can develop in children as well as adults. However, the disease seems to be different from adults requiring a more conservative approach to management. It is less commonly seen than adults and appears to be less aggressive, being characterized by a variable outcome reported in literature from aggressive course and a high mortality to less aggressive course and even spontaneous regression. We reported the case of idiopathic myelofibrosis in the early childhood of a boy successfully treated with standard doses of steroid. A 4 month-old boy was admitted because of severe anemia with reticulocytopenia, aniso-poikilocytosis, leukoerythroblasts, teardrop-shaped red cells and sphenomegaly. The marrow was very difficult to aspirate. The bone marrow biopsy revealed reticulin myelofibrosis. Cyrogenetic study of the marrow was negative. The condition worsened with development of severe thrombocytopenia. Investigations done repeatedly ruled out malignant hemopathy, metastatic infiltration of the marrow, myelodysplasia, osteopathy, lupus erythematosus, immune disease and Fanconi anemia. After 5 months and 3 red cells transfusion prednisone therapy was attempted at 2 mg/kg/d. A complete improvement of hematological and clinical findings was observed after a month and a half. He is now 13 months old on 15 mg/d of steroid with a hemoglobin of 14g% and platelets around 410000/mm3.

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QT DISPERSION AFTER ADMINISTRATION OF RAPID INTRAVENOUS VARIOUS ANTIEMETICS IN CHILDREN PRIOR TO CHEMOTHERAPY
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Backgrounds. Children with acute leukemia are at an increased risk of cardiac arrhythmias, from their cardiac infiltrations and cardiotoxic treatments. There are many reasons why the children with acute leukemia is at increased risk of potentially life-threatening cardiac arrhythmias. The autonomic response to chemotherapy and radiation therapy (nausea, retching and vomiting) or their biochemical ejects (vomiting-induced electrolyte disturbance) can have important implications. It is, therefore, important to ensure that any medications to administered to the children, do not further increase the risk of cardiac complications, particularly arrhythmias. Nausea and vomiting are considered to be the most distressing and debilitating side effects of therapy, and can profoundly affect patients’ quality of life. Aim and Methods. The aim of this study was to determine the effect of the rapid administration of intravenous tropisetron, granisetron and ondansetron on measures of cardiac depolarization in children receiving chemotherapy for acute leukemia, by comparing twelve-lead ECGs before (baseline) and after 2nd and 24th hours after the drug administration. Results. The study was performed in total 75 children with acute leukemia (25 children for each antiemetic). QT dispersion was calculated as the difference between the maximum and minimum QTc in twelve-lead surface electrocardiogram lead. Summary/Conclusions. It was concluded that no clinically important cardiac vascular side effects are associated with the administration of tropisetron, granisetron and ondansetron following first 24 hour. There are no dysrhythmic or hemodynamic changes in all patient groups.

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EFFECT OF RECOMBINANT ACTIVE FACTOR VII ON VOLUME OF BLOOD TRANSFUSION AT PATIENTS UNDER CPB WITH NOT SURGICAL BLEEDING
M. Charnaya
Russian Research Center of Surgery, MOSCOW, Russian Federation

Backgrounds. to estimate effect rFVIIa on reduction of blood transfusion at patients under CPB with not surgical bleeding. Materials and methods. We surveyed 60 pts under CPB with diffuse bleeding 9,0±3,3 ml/mes (from 3,0 up to 20,0 ml /mines); group 1 (n=34) - single bolus administration rFVIIa i.v. 7,5±10,1 mg/kg of weight; group 2 (n=26) - group of comparison. Estimated volumes of autoblood (ml), donor erythrocytes (ml), washed red autoblood cells (ml), fresh-frozen plasma (ml) separately before use of a preparation. Results. In group 1 through 30 min after administration rFVIIa bleeding has essentially decreased, and in 2 hours it has completely stopped. In group 2 bleeding was kept till 12 hours after operation. Up to administration rFVIIa autoblood it was poured 11% of pts, and after administration - 0%, red blood cells - 67 and 59%, washed erythrocytes - 33 and 0%, FFP - 78 and 50%, respectively. In 55% of cases was not required any blood transfusion. Authenticated distinctions in quantities autoblood (795,0±21,7 and 906,7±45,8 ml, p<0,05), red autoblood cells (354,3±94,5 and 881,2±82,6 ml, p<0,05) and washed erythrocytes (295,6±122,2 and 611,4±93,7 ml, p<0,001) between groups 1 and 2 are revealed. Conclusion: Use rFVIIa in therapy of uncontrollable not surgical bleedings at CPB results in significant reduction of frequency of use and volumes blood transfusion.

1476
ANALYSIS OF THE EFFECTIVE AGENTS IN EPITHELIS C VIRUS INFECTION AMONG HEMOPHILIC PATIENTS TREATED IN HEMOPHILIA CENTER OF ISFAHAN-IRAN
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Isfahan University of Medical Sciences, ISFAHAN, Iran

Backgrounds. Patients with hemophilia are at high risk of post-transfusion Epstein Barr virus because of widespread use of plasma-derived products. As a consequence, hepatitis C virus (HCV) is the most common cause of chronic liver disease among hemophilic patients. Aim: The objectives of this study are to determine HCV prevalence, and analyze the effective agents in HCV infection in hemophilic patients. Method: all patients with inherited coagulation disorders registered in hemophilia center of Isfahan (885 persons) were checked for HBsAg and anti-HCV, using a third-generation enzyme-linked immunosorbent assay (ELISA) test. Positive tests for anti-HCV were confirmed by RT-PCR. Clinical history, Laboratory and treatment data of all cases were studied in January 2006. Results. From 465 men and 88 women with inherited coagulation disorders with Mean±SD age of 23.4 ±12.9 years,125 patients (22.6%) had HCV positive, 2 (0.4%) were HBV positive and one(0.2%) was both HBV and HBV positive. In this study the chance of coloration (with percentage correct of 72.4%) between HCV infection and cryoprecipitate usage was 5.31, between HCV and FFP usage was 3.16 and between HCV infection and moderate and severe hemophilia were 3.9 and 2.65 respectively. In this study blood group, factor concentrate consumption, age and sex of patients have no predictive value in HVC infection. 44.4% of patients with factor inhibitor were HVC positive. (p=0.006). Conclu- sion: Considering the high chance of HCV infection after transfusion of Cryoprecipitate and FFP, a more careful pre-transfusion screening of blood for anti-HCV must be introduced in all blood banks. The usage of FFP which has less chance of HVC infection, instead of cryoprecipitate in patients who do not have volume restrictions may be preferable.

1477
CLINICAL AND EPIDEMIOLOGICAL CHARACTERISTICS OF THROMBOCYTOPATHIES IN CHILDREN IN KAZAKHSTAN
G.T. Tashenova, B.Z.H. Aldaniarova, K.O. Omarova
Scientific Center of Pediatrics and Chil. ALMATY, Kazakhstan

The problem of bleedings in childhood is one of the most actual. Thrombocytopenias (TP) - the group of diseases characterized by platelet dysfunction with predominantly microvascular type of bleeding. In structure of hemorrhagic diatheses TP has leading position (80%). The aim of this study was to assess the clinical and laboratory peculiarities of the 105 children hospitalized in hematooncological department of the Scientific center of pediatrics and children's surgery. Diagnostic complex was included anamnestic data, duration and character of the bleeding, platelet number and coagulation. Genealogical anamnesis on bleeding was aggravated in 75% of patients with predominantly autosomal-dominant inheritance. In 15 patients parents were examined. Hereditary TP was diagnosed in 95 (91%) patients, acquired TP in 10 (9%), out of them: drug-induced in 4 (3,5%), post-infectious in 4 (3,5%), due to endocrinopathy (hypoestrogenia) in 2 (1%). Hemorrhagic syndrome was primarily diagnosed in earlier childhood in 57 patients (54%), frequency of the relapses was increased to 10-12 y.o. in 77 patients (72%), to 15-16 y.o. bleeding symptoms regressed. Patients were divided into groups: I group - with deficit of plasma adhesion and aggregation factors (von Willebrand disease, vWD and a fibrinogenemia, aF) and II group - with platelet factors alterations (inherited TP). Patients of the I group had complaints: nasal bleedings (58%) and skin hemorrhages (54%). Patients of the II group had less complaints: nasal bleedings (38%)

haematologica/the hematology journal | 2006; 91(s1) | 521
or skin hemorrhages (24.3%), in 43% children were directed by other specialists due to petechia appearing after procedures, anemia or others. Perinatal pathology (skin hemorrhagic syndrome, umbilical hemorrhages, intranatal intraventricular hemorrhages) was more frequent in I group (54.5%) in comparison with II group (27.5%). Bleeding time was increased in 98.8% of patients. Alterations in adhesion and aggregative platelet functions were noted in 57.4% (ristocetin-induced platelet aggregation decreased in 26% of patients with normal platelet number, decreased adrenalin-induced aggregation in dose 2.5 mg/ml, adrenalin-induced aggregation in dose 1 mg/ml was established in 19% of patients. After assessment of clot retraction, the decreasing lower than 40% was noted in 4 patients, and Glanzmann's thrombostena diagnosis was established. The decreasing of the secondary aggregation (second wave) induced with adenosine-diphosphate and adrenalin was established in 38% of patients. The diagnosis of von Willebrand disease was established in 26% of patients with normal platelet number, increased activated partial thromboplastin time (over 55 sec), decreased activity of von Willebrand factor (lower than 80%). Neurologist has established vegetative vascular dystonia and intracranial hypertension in 12 patients. In 11 patients otolaryngologist has revealed during the rhinoscopy the superficial localization of blood vessels, especially in Kesselbakh zone. Thus, nasal bleedings in children with thrombocytopathias - the most often symptom. Bleeding in form of petechias, ecchimoses, gingival, nasal bleeding in children with normal or slightly decreased platelet number may be due to qualitative disorders of platelet aggregation in form of hematomas, hemorrhorrhages reveals double defect in hemostasis, typical for von Willebrand disease. After establishment of bleeding type laboratory investigations to reveal character of hemostasiopathy are necessary. Vegetative vascular dystonia, intracranial hypertension, vascular changes in nasal mucosa, possibly, may play role in pathogenesis of nasal hemorrhages in patients with hemostasis disorders. Earlier establishment of the cause of nasal bleedings will allow to conduct an adequate therapy.

1478
NASAL BLEEDING IN CHILDREN WITH HEREDITARY THROMBOCYTOPATHIAS
B.Z.H. Aldamarova, K.A. Mazhibayev, G.T. Tashenova, K.O. Omarova
Scientific Center of Pediatrics and Children, ALMATY, Kazakhstan
Nasal bleeding in children is not rare pathologic condition, which causes diagnostic and therapeutic difficulties among physicians. Bleeding from nasal cavity is not a disease, but the symptom of the local or systemic disease. The most intensive and severe nasal bleeding more often has place in cases of hereditary thrombocytopathia. Hemorrhagic diatheses are characterized by hereditary, congenital or acquired system bleeding disorders. According on data of various investigators, from 40% to 80% of all cases of bleeding disorders related with quantitative and qualitative disorders of thrombocytopathia. Among thrombocyte-dependent hemostasis disorders the special interest is directed to thrombocytopathies. In fact that 80% of hemorrhagic diatheses are related with disorders of primary (thrombocytopathic) stage of hemostasis. However, the clinical manifestations of the majority of hemostasiopathies are monotypic, which makes the diagnostic difficulties. Earlier establishment of the cause of hemostasis disorder is necessary for administration of an adequate hemostatic therapy. Under our observation were 42 children aged from 1 to 15 years old with thrombocytopathies, hospitalized to oncohematologic center in SCPCS. The diagnosis was based on anamnesis, clinical manifestation, laboratory data. Assessment of hemostasis system was based on the results of standard investigations: platelet number, functional activity of platelets: adhensive and aggregative functions in vitro and aggregometry. Investigation of factor von Willebrand activity was conducted in ‘HEM’ company. Moreover, all children were consulted by neurologist and otolaryngologist. In result of the analysis the following types of hemostasiopathies were revealed: releasing thrombocytopathias in 27 (64%) patients, von Willebrand disease in 11 (26%) and Glanzman's thrombastenina in 4 (10%). Of them in 31 (74%) patients relapsing nasal bleeding was noted. The first bleeding manifestation in 17 (40,5%) was noted from the birth with the high frequency of bleeding up to 3 years old. The majority - 24 (57%) of patients were boys. Hereditary character of the disease was in 76%, acquired forms was established in 24% of patients. In majority of cases nasal bleeding was combined with other localization (petechias, ecchimoses, gingival bleeding, metrorrhages, post-traumatic and post-injection bleeding, bleeding from ears and hemorrhages into sclera). Hematomas and hemarthrosis typical for hematogenic type of bleeding disorders were noted in 2 patients with von Willebrand disease. In result of laboratory investigations normal number of platelets was noted and this indicator doesn't change during the study period. An assessment of Duke’s bleeding time showed prolongation in 85% of patients (more than 4 min). The time of coagulation in all patients was normal 96-10 min). Prolongation of bleeding time was seen in cases of hemorrhagic diatheses, which was typical for hematogenic type. To reveal platelet dysfunction we have assessed an adhesive function of platelets. An adhesion of platelets to glass was assessed in 21 children, out of them in 18 (85,7%) adhesive dysfunction was revealed and

was 0.20% in comparison with normal 30-40%. To exclude coagulative disorders we have assessed standard indicators of hemostasis system: coagulation time, prothrombin time, activated partial thromboplastin time, thrombin time, blood fibrinogen level, von Willebrand factor. Generally, all those indicators was in normal range. An assessment of aggregative function of platelets showed decreased aggregation to ristocetin in 19% patients and an absence of aggregation in 7%. An assessment of platelet functions revealed, large aggregation in 54,5% in comparison with II group (27,3%).
involved in the control of the immune system. This review focuses on recent advances in the understanding of the immune system and its role in health and disease. The immune system is a complex network of cells, molecules, and tissues that work together to defend the body against pathogens, such as viruses, bacteria, and fungi, and to maintain tissue homeostasis.

The immune system consists of two main branches: the innate immune system and the adaptive immune system. The innate immune system provides a non-specific, rapid response to infection, while the adaptive immune system is a specific, slower response that is directed against specific pathogens.

The innate immune system includes phagocytes, such as macrophages and dendritic cells, which engulf and destroy pathogens. It also includes natural killer cells, which can kill virus-infected cells and cancer cells. The adaptive immune system includes B cells, which produce antibodies that can neutralize pathogens, and T cells, which can directly kill infected cells and regulate the immune response.

The immune system is also involved in the regulation of inflammation, tissue repair, and tolerance to self-antigens. Dysregulation of the immune system can lead to autoimmune diseases, allergies, and infections.

Understanding the immune system is crucial for the development of effective vaccines and treatments for a wide range of diseases. Future research in this area is likely to focus on improving our understanding of the molecular mechanisms that underlie immune function and on developing new strategies for the treatment of immune-related disorders.

References


quickly with concomitant HbS removal and ameliorating hyperviscosity. We report about SCD acute painful crisis in a 25-years-old woman, who benefit by automated TREX performed at our Apheresis Service using Fresenius™ COM.TEC device with a new dedicated program.

Table 1. Characteristics of the patient.
<table>
<thead>
<tr>
<th></th>
<th>1st TREX</th>
<th>2nd TREX</th>
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<tbody>
<tr>
<td>Pain (black, abdominal, legs)</td>
<td>intense</td>
<td>reduced</td>
</tr>
<tr>
<td>Morphine</td>
<td>i.v. continuously</td>
<td>suspended no</td>
</tr>
<tr>
<td>Hct (%)/Hb (g/dL)</td>
<td>26.0/9.2</td>
<td>27.8/9.6</td>
</tr>
<tr>
<td>Hbs (%)</td>
<td>80</td>
<td>28</td>
</tr>
<tr>
<td>Mean Ht(%)</td>
<td>56</td>
<td>57</td>
</tr>
<tr>
<td>Patient blood volume (mL)</td>
<td>3710</td>
<td>3710</td>
</tr>
<tr>
<td>Processed blood volume (ML)</td>
<td>3997</td>
<td>3329</td>
</tr>
<tr>
<td>Flow rate (mL/min)</td>
<td>37</td>
<td>40</td>
</tr>
<tr>
<td>Time of procedure (min)</td>
<td>112</td>
<td>93</td>
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</table>

Methods: a blood specimen was drawn in advance to assess compatibility, then cross-matched RBC units were filtered for leukocyte depletion. A detailed informed consent was obtained. Erythroexchange was performed by double-vein technique using a new program (Fresenius HemoCareTM, Bad Homburg, Germany) which permits to predict both the final hematocrit and HbS level of the patient. Blood cell count and HbS percentages by current haemoglobin electrophoresis were measured before and after each TREX. The pre-apheresis HbS value permitted to appropriately set the cell separator program with the goal of reducing HbS to less than 30%. Check of post-apheresis HbS allowed verifying the accuracy of instrument predictions. During the procedure the patient was carefully monitored as respect to vital signs (blood pressure, heart rate, oxygen saturation) and occurrence of adverse events, in particular signs of transfusion reaction. Calcium gluconate was administered i.v. to prevent or minimize citrate toxicity. Results: we performed two TREX procedures on alternate days; the relevant data are given in the table 1 and 2. Complete clinical remission was obtained with no evidence of alloimmunization or other serious complications. Conclusions: our experience confirms the beneficial effects of TREX for SCD pain crisis especially when isovolumetric procedures are carried out with an automated device.

Table 2. Characteristics of erythroexchange.
<table>
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<tr>
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<th>1st TREX</th>
<th>2nd TREX</th>
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<tr>
<td>No./total volume of RBCs* (mL)</td>
<td>8/1955</td>
<td>7/1823</td>
</tr>
<tr>
<td>Mean Ht(%) of RBCs*</td>
<td>56</td>
<td>57</td>
</tr>
<tr>
<td>Patient blood volume (mL)</td>
<td>3710</td>
<td>3710</td>
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<td>Time of procedure (min)</td>
<td>112</td>
<td>93</td>
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RBCs* = red blood cell units

Formed. Measured were WBC, MNC and CD34+Cells in the peripheral blood, amount of CD34+ cells /kgBW in the apheresis products, collected CD34 cells/processed liter and efficacy of the procedures (MNCs, CD34+ cells.) Results: According to the specifications of the transplanting departments (CD34 cells > 2x10^6/kgBW + back up) 190 (73.8%) of the aphereses were completed successfully, 31 out of 190 (16.3%) successfully completed aphereses were started with CD34+cells<20/µL. In 19 out of 25 aphereses we were successful with CD34+cells <20/µL and >0.2%. Conclusion: In collections started with CD34+cells <20/µL the percentage of CD34+ cells is a good predictive factor for successful apheresis, even more so for adults then for children. So don’t forget to look at the percentage of CD34+ Cells in peripheral blood when you decide to start with stem cell apheresis.

Table 1.

<table>
<thead>
<tr>
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<th>CD34+ &gt;0.2%</th>
<th>CD34+ &lt;0.2%</th>
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<tbody>
<tr>
<td>Adults</td>
<td></td>
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<tr>
<td>&gt;20/µL</td>
<td>97% (104/107)</td>
<td>76% (23/30)</td>
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<tr>
<td>&lt;20/µL</td>
<td>82% (14/17)</td>
<td>13% (7/53)</td>
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<tr>
<td>Children</td>
<td></td>
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<tr>
<td>&gt;20/µL</td>
<td>95% (20/21)</td>
<td>92% (12/13)</td>
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<tr>
<td>&lt;20/µL</td>
<td>83% (5/5)</td>
<td>41% (5/12)</td>
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Thrombotic thrombocytopenic purpura (TTP) is a rare complication of hematopoietic stem cell transplantation (HSCT): the literature is scant and heterogeneous, little is known about the pathogenesis, except that it appears to differ from that of classical TTP. Plasma exchange (PE) is commonly employed for the therapy, but there are no data that support its use. We present our experience in treatment of two post-HSCT TTPs with PE. From May 2004 to December 2005, 52 patients underwent HSCT, and TTP was diagnosed in 2 of them, respectively on post-transplant day 47 and 102. Both patients received HSCT from HLA-compatible related donors. TTP was defined as the simultaneous occurrence of red cell fragmentation, laboratory findings of haemolysis with negative direct and indirect antiglobulin test, high LDH level, red cell transfusion requirement and thrombocytopenia caused by consumption, in the absence of disseminated intravascular coagulation. PE was performed using fresh frozen plasma as replacement fluid. PE was well tolerated, but the two patients had no response to the treatment. One patient died because of fungal infections. Our experience confirm the data of the recent literature. TTP is a rare and serious complication of hematopoietic stem cell transplantation and further, systematic studies are necessary for a better knowledge of its incidence, treatment and outcome.

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PREHARVEST PREDICTIONS OF STEM CELL PRODUCTS

R. Gilli, S. Sipurzynski, S. Machek, K. Rosskopf, W. Helmberg, G. Lanzer
Univ. Klinikum Graz, GRAZ, Austria

Backgrounds. Collected mobilized peripheral stem cell is the commonly used resource for autologous transplantation. The amount of CD34+ cells in the peripheral blood is used as determinant for starting collection. Aims. Aim was to see if not only the amount of CD34+ cells /µL determines the yield of the product but also the percentage of CD34% cells should be considered when to start with collection. Methods. 259 aphereses of adult patients suffering from hematological (AML, CML, NHL, MM) disorders and children with solid tumors (ewing sarcoma, neuroblastoma, rhabdomyosarcoma,) were evaluated. Aphereses were done with the Cobe Spectra™, 3-4 times the blood volume was processed. Measured were WBC, MNC and CD34+Cells in the peripheral blood, amount of CD34+ cells /kgBW in the apheresis products, collected CD34 cells/processed liter and efficacy of the procedures (MNCs, CD34+ cells.) Results: According to the specifications of the transplanting departments (CD34 cells > 2x10^6/kgBW + back up) 190 (73.8%) of the aphereses were completed successfully, 31 out of 190 (16.3%) successfully completed aphereses were started with CD34+cells<20/µL. In 19 out of 25 aphereses we were successful with CD34+cells <20/µL and >0.2%. Conclusion: In collections started with CD34+cells <20/µL the percentage of CD34+ cells is a good predictive factor for successful apheresis, even more so for adults then for children. So don’t forget to look at the percentage of CD34+ Cells in peripheral blood when you decide to start with stem cell apheresis.

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<tr>
<td>&lt;20/µL</td>
<td></td>
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<tr>
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<td>&gt;20/µL</td>
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<td>&lt;20/µL</td>
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PREHARVEST PREDICTIONS OF STEM CELL PRODUCTS AIMS. To verify the applicability of the Czech version of an questionnaire for determining the yield of the transplantable stem cell products and efficacy of the procedure. Methods. Aims. 1. to verify the applicability of the Czech version of an international generic European Quality of Life Questionnaire - Version EQ-5D for the evaluation of QoL in patients after the HSCT at the Department of Clinical Hematology of the 2nd Internal Clinic of the University Hospital and Medical Faculty of Charles University in Hradec Králové, Czech Republic.
QUALITY OF LIFE OF LITHUANIAN CHILDREN SUFFERING FROM CANCER

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Cancer is the most often cause of death in children. According to data of the Lithuanian cancer registry, in the last decade 70'100 new cases of childhood cancer were diagnosed yearly. In Lithuania, the quality of life of children suffering from cancer until now has not yet been evaluated properly. Aims. The aim of the study is to increase the understanding of the quality of life of Lithuanian children suffering from cancer. Methods. The study started in 2005 in the Division of Oncology and Hematology at the Clinical Hospital of Kaunas University of Medicine and in the Division of Oncology and Hematology at the Vilnius University Children’s Hospital. During one year, 63 children aged 2-18-year and their families were invited to participate in the study. In the sample, 55% of children suffered from hematoblastosis, 13% from CNS tumors, and 32% from solid tumors of other localizations. The children and their parents were questioned within 2 to 6 weeks from the date of diagnosis. We used the PedsQL (Pediatric Quality of Life Inventory TM) questionnaire initially developed to evaluate the quality of life of children between the ages of 2 and 18. The questionnaire was translated from the original English version (designed by Dr. James Varni and the Mapi Research Institute) into Lithuanian according to the linguistic validation criteria. Children aged 5'7 were interviewed by researchers while older children and parents of children from all age groups filled out the questionnaires by themselves. Results. 36.1% of children aged 8'18 stated that they had low energy often or almost always; 54.9% of their parents thought similarly. Among the 5'7 children complained of having low energy when compared with older children; none of the children in this age group complained of having always being tired. 40.0% of parents whose children were 2'4-year-old felt that children often or almost always needed more energy to play. Among 8'18-year-olds, 27.8% of respondents stated they never felt scared and sad; 22.2% of the respondents did not feel angry because of their present disease. In this age group, 9.7% and 16.1% of parents felt scared, sad and angry respectively. 33.3% of respondents stated that they sometimes felt uneasy that their disease will relapse; parents worried about this more often - 41.9%. Furthermore, in the 8'18-year age group, 27.8% of children stated feeling pain often or almost always, whereas this complaint was stated by 38.8% of their parents. Among 5'7-year-olds, often or almost always felt pain was reported by 12.0% of children, whereas the child’s pain was indicated by 28.9% of parents. Among 2'4-year-old children, 46.7% of parents stated that their children often felt or almost always felt pain. Conclusions. Children evaluated their quality of life as being better when compared with their parents. Younger children evaluated their quality of life as better than older children.

THROMBOPHILIC MUTATIONS IN THALASSEMIA AND β-TALASSEMIA

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University Hospital of Ioannina, IOANNINA, Greece; 2Dept. of Haematology, IOANNINA, Greece

Backgrounds. The thalassemic patients present a higher than normal incidence of thromboembolic events including cerebral thrombosis, deep veinous thrombosis and pulmonary embolism. The study of the homo- and heterozygotes of the thalassemic patients revealed increased circulating platelet aggregates, a significant shortening of platelet life span, increased concentrations of urinary metabolites of thromboxane A2 and prostacyclin, low levels of protein C and protein S. On the other hand, a number of mutations are associated with an increased risk of thrombosis. Heterozygotes for the Factor V G1691A (Leiden) mutation experience 2-5 times the normal risk of thrombosis, while homozygotes’ risk is 80 times the risk in non carriers. The MTHFR C677T and A1298C mutations are associated with homocysteinemia and increased risk of cerebrovascular disease and peripheral artery disease. Other thrombophilic mutations are the Factor V G1691A (Leiden), Prothrombin G20210A, β-Fibrinogen 455 G-A, PAI-1 4G-5G, GPIIa L33P (HPA-1), ACE I/D, Apo B R3500Q and Apo E2/E3/E4. Aims. The aim of the present study was to check whether the presence of a thrombophilic mutation in a thalassemic patient increases the risk for the development of a thromboembolic event.

Table 1. Number and percentage of thrombophilic mutations.

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Thalassemic (20)</th>
<th>Non Thalassemic (20)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>Factor V G1691A</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Factor V H1299(R2)</td>
<td>20</td>
<td>3 (15%)</td>
</tr>
<tr>
<td>Prothrombin G20210A</td>
<td>4 (20%)</td>
<td>20</td>
</tr>
<tr>
<td>Factor XIII V34L</td>
<td>20</td>
<td>11 (55%)</td>
</tr>
<tr>
<td>B-Fibrinogen 455 G-A</td>
<td>20</td>
<td>11 (55%)</td>
</tr>
<tr>
<td>PAI-1 4G/5G</td>
<td>20</td>
<td>15 (75%)</td>
</tr>
<tr>
<td>GPIIa L33P (HPA-1)</td>
<td>20</td>
<td>1 (5%)</td>
</tr>
<tr>
<td>MTHFR C677T</td>
<td>20</td>
<td>8 (40%)</td>
</tr>
<tr>
<td>MTHFR A1298C</td>
<td>20</td>
<td>7 (35%)</td>
</tr>
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</table>

Methods. We have screened an unselected group of 20 patients, 17 men and 3 women. 16 of them had β-thalassemia major, 2 intermediate β-thalassemia and 2 S-β-thalassemia. The mean age of the patients was 30.5 years. One of the patients presented ulcer of the lateral malleolus, another had avascular necrosis of the femoral head. A group of 20 sex- and age-matched healthy individuals served as control group. DNA analysis was performed by polymerase chain reaction and reverse hybridization. Both patients and healthy individuals were checked for 9 mutations: FV G1691A (Leiden), FV H1299(R2), Prothrombin G20210A, Factor XIII V34L, β-Fibrinogen 455 G-A, PAI-1 4G/5G, GPIIa L33P (HPA-1), ACE I/D, Apo B R3500Q and Apo E2/E3/E4.
intermediate \( \beta \)-thalassemia and ulcer of the lateral malleolus was heterozygous for FXIII V34L, \( \beta \)-Fibrinogen '455 G-A, PAI-1 4G/5G, MTHFR C677T. The 57-year old man with \( \beta \)-thalassemia and avascular necrosis of the femoral head was heterozygous for prothrombin G20210A, FXIII V34L, \( \beta \)-Fibrinogen '455 G-A, PAI-1 4G/5G, MTHFR A1298C. Conclusions. The prevalence of the thrombophilic mutations in thalassemic patients doesn’t differ much of the prevalence of these mutations in non thalassemic people. However, the presentation of any of the thrombophilic mutations in a thalassemic patient is a factor that contributes, among others, to the development of a thromboembolic event.

1489 AUDIT OF INDICATIONS FOR OUT OF HOURS COAGULATION SCREENING AT THE ULSTER HOSPITAL

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The number of coagulation screens performed out of hours has rapidly been increasing, with a corresponding rise in the cost to laboratories, both in terms of materials and staff time. In January 2005, almost 700 coagulation screens were performed out of hours. It was felt that many of these were not clinically indicated, and most were normal. Of the abnormal results, only a small percentage were actually treated. It was therefore felt that an audit in this area was appropriate, to examine how this resource is being misused. The aims of the study were as follows: - to study the out of hours coagulation screens performed in the Ulster Hospital; to produce guidelines to be followed prior to performing the test; to rationalise the number of tests performed. 100 patients who had coagulation screens performed out of hours in January 2005 were randomly selected. The indications for the test were examined; appropriate indications included known or suspected liver disease, history of haemorrhage, current haemorrhage or renal failure. We also looked at treatment given for abnormal results. We felt that treatment should be given if the result was abnormal by 50% or more. The total number of screens performed in the one month period was 677, taken form 592 patients. Of the 100 cases we examined, 70% were in fact normal. Only 35% of the tests were clinically indicated. Only 10% of the patients who had abnormal results were treated. We concluded from the study that the majority of coagulation screens performed out of hours in the Ulster Hospital were not indicated; even if the results were abnormal, most were not acted upon. We therefore feel that local and national guidelines ought to be developed, to reduce wastage of this resource.

1490 PRE-EMPTIVE ANTIFUNGAL THERAPY IN HIGH-RISK PATIENTS WITH ACUTE LEUKEMIA: COST-EFFECTIVENESS OF INTRAVENOUS ITRACONAZOLE

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Backgrounds. Systemic fungal infection remain a major clinical problem in immunocompromised patients, particularly in patients with prolonged severe neutropenia, preexisting myelodysplasia and advanced age. In these cases, presumed systemic fungal infections are treated empirically to reduce documented infections and associated mortality. Aims. We, retrospectively, compared the cost-effectiveness of intravenous itraconazole treatment with conventional treatment with liposomal amphotericin-B or new antifungal drug as voriconazole, in patients affected by AML or high-risk MDS. Methods. Since January 2003 to December 2005, 38 patients (24 female and 14 male, median age 72) affected by AML or high-risk MDS, who underwent induction chemotherapy, received primary antifungal prophylaxis. Twenty patients were treated with fluconazole 200 mg/day os and 18 patients with itraconazole 200 mg bid os. During induction therapy the median length of severe neutropenia (PMN<500/m3) was 19 days. Febrile episodes have been empirically treated with broad-spectrum antibiotics (cephalosporine plus aminoglicoside with or without glycopeptide). Pre-emptive and empirical antifungal treatment for fever unresponsive to broad-spectrum antibiotic therapy was employed, after 5 days, in 20 fluconazole patients with liposomal amphotericin-B 3 mg/kg/die intravenous in 9 patients or voriconazole 6 mg/kg bid during first 24 day followed by 4 mg/kg bid intravenous in 11 patients. In the subgroup treated with oral itraconazole all patients were switched to intravenous drugs at the dose of 200 mg over 60 minutes every 12 hours during the first 2 days followed by 200 mg given i.v. once daily. All patients were treated with pre-emptive therapy for a median of 14 days (11-21). Results. There were no significant differences noted between the three subgroups with regard to the duration of prophylaxis (median: 10 days for fluconazole vs 11 days for oral itraconazole), percentage of patients who developed fever unresponsive to broad-spectrum antibiotic therapy (46% in fluconazole group vs 39% in itraconazole group), proven/probable or possible fungal infection as well as with regard to survival. Safety and toxicity analysis of pre-emptive treatment was similar in all subgroup, only one patients withdrawal from voriconazole therapy for hallucination and one withdrawal from intravenous itraconazole for nausea and vomiting therapy resistant. When we compared cost of three pre-emptive therapy we showed that lipid amphotericin-B and voriconazole were most expensive than intravenous itraconazole both we consider daily therapy cost and total treatment associated with nurse cost. Conclusion: Intravenous itraconazole has at least equivalent efficacy as empirical antifungal therapy in immunocompromised patient affected by AML or high-risk MDS. However, intravenous itraconazole compared with other antifungal treatment was shown to be the best cost-effective and cost-saving pre-emptive empirical therapy.
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